Effect of Different Conditioning Protocols on Adhesion of a GIC to Dentin


Purpose: This study examined the ultrastructure and microtensile bond strength (μTBS) of a restorative glass-ionomer cement (GIC; Dentsply) to sound dentin that was conditioned with various techniques.

Materials and Methods: Dentin surfaces from extracted human third molars were abraded with 180-grit SiC paper. Five groups of three teeth each were prepared: C - no acid pretreatment (control); P - 10% polyacrylic acid (PAA) for 10 s, no rinsing; R - 10% PAA for 20 s, rinsed; K - 25% PAA for 25 s, rinsed; and H - 32% phosphoric acid for 15 s, rinsed. TEM was performed on a bonded specimen from each group, using unstained, undemineralized sections. GIC buildups were made on the remaining teeth, and after storage at 100% humidity for 24 h, the teeth were sectioned for μTBS and SEM evaluation.

Results: TEM revealed the presence of a structure known as the intermediate layer in all groups. This layer contains metallic salts contributed by both the GIC and dentin. In group C, this layer was restricted to the smear layer. In groups P and R, intermediate layers could be found above partially demineralized zones within the intertubular dentin. In groups conditioned with more aggressive protocols (K and H), the intermediate layers shifted downward to reside within the superficial portions of completely demineralized collagen. Group C had statistically lower μTBS (p < 0.05), while the other groups were not significantly different from each other. SEM revealed adhesive failures along the dentin surface in group C and mixed failures in the other groups.

Conclusion: The lower μTBS observed in the control group reflects the weakness of the smear layer attachment to dentin. The higher μTBS in the other groups probably represent the cohesive strength of GIC under tension, rather than its true adhesive strength to dentin. Acid pretreatment dissolves the smear layer, creates a zone of partially demineralized dentin, and allows the PAA to interact with dentin via the intermediate layer. Overly aggressive conditioning renders the dentinal tubules patent, and leaves deeper demineralized dentin that does not form part of the intermediate layer.

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Conventional chemically-cured (ie, acid-base reactions) glass-ionomer cements (GICs) were developed almost three decades ago at the Laboratory of Government Chemists in England. As a restorative material, GICs have been successfully used for the restoration of cervical lesions. However, poor fracture and wear resistance and lack of radiopacity made early generation chemically-cured GICs problematic as permanent load-bearing restorations. This is also true of the subsequently improved, more viscous formulation. High strength GICs that were recently developed for atraumatic restorative treatment (ART) of posterior teeth exhibit better flexural...
strength and produced satisfactory three-year clinical results at least for single-surface restorations.\textsuperscript{12}

To circumvent surface contamination by smear layers produced during tooth preparation, different conditioners have been investigated to enhance the laboratory adhesion of GICs to enamel and dentin, with equivocal results.\textsuperscript{27,29,41} Most manufacturers now recommend the use of polyacrylic acid (PAA), with variable concentrations and etching times, as GIC pretreatment protocols. Being a weak acid, PAA leaves smear plugs intact,\textsuperscript{14} prevents ingress of bacteria via exposed dentinal tubules, and dilution of the setting GICs via transudation of pulpal fluid.\textsuperscript{37} Studies that evaluated the efficacy of PAA pretreatment have also produced contradictory results.\textsuperscript{2,16} This may be related, in part, to the thickness of the smear layers employed in different studies.\textsuperscript{27} The controversy is also complicated by the repeated observations of cohesive cement failures instead of interfacial adhesive failure in bonded GICs.\textsuperscript{23,37} The low bond strength values of GICs reported using conventional shear and tensile tests\textsuperscript{4,7,40} may thus represent the low cohesive strengths of the cements rather than their true adhesive strength to dentin.

Although GICs bond chemically to tooth substrates, their bonding mechanism is complex and is not completely understood.\textsuperscript{22} On placement, the hydrophilic, aqueous polyalkenoic acid wets the enamel and dentin, providing intimate contact with the cement. The proposed mechanism involves carboxylic groups of the polyelectrolyte replacing phosphate ions of the substrate to establish ionic bonds with the calcium ions derived from partially dissolved apatite crystallites.\textsuperscript{44} There is also initial evidence suggesting that chemical bonding of PAA may also occur with collagen.\textsuperscript{21} Ion exchange and reprecipitation of ions released by the glass particles and those dissociated from the partially demineralized dentin\textsuperscript{33,42} probably account for the SEM observation of a fluoridated intermediate layer,\textsuperscript{13} an interaction zone,\textsuperscript{20} an interdiffusion zone,\textsuperscript{8} or the presence of an interphase\textsuperscript{33} between GIC and dentin. Although the glass-hydrogel interface in GICs have been documented for some time using transmission electron microscopy (TEM),\textsuperscript{15} ultrastructural characterization of the GIC-dentin interface remains elusive. The use of acidic conditioners for GIC pretreatment, irrespective of whether or not they are rinsed away before bonding, invariably results in some demineralization of the intact dentin. It is not clear how the intermediate layers in water-based GICs differ from micromechanically retentive hybrid layers that are produced by contemporary resin-based, self-etching and total-etch adhesives.

This study evaluated the ultrastructure of the GIC-dentin interface when a chemically cured (ie, acid-base reaction) GIC was bonded to coronal dentin. To evaluate how GICs interact with the bonding substrate, different pretreatment protocols were used, comprising PAA acids of variable concentrations and etching times and phosphoric acid. This produced a series with progressively aggressive etching effects on the smear layer-covered dentin. Ultrastructures of the GIC-dentin interfaces that were tested using the non-trimming version of the microtensile bond test\textsuperscript{34} were further correlated with bond strength. Bonded specimens that were stressed to failure were also examined with cryo-SEM to determine whether cohesive failures in GIC were minimized when the GIC was used for bond testing. The null hypothesis was that the ultrastructure of the GIC-dentin interface produced by the various pretreatment protocols does not correlate with their tensile bond strengths to dentin.

**MATERIALS AND METHODS**

**Tooth Preparation**

All bonding was performed on the occlusal surfaces of mid-coronal dentin of 15 extracted, human third molars from young adults of both genders. They were stored in 0.5% chloramine T at 4°C and used within one month following extraction. The teeth were prepared by removing all of the occlusal enamel using a slow-speed saw with a diamond-impregnated disk (Isomet, Buehler, Lake Bluff, IL, USA) under water lubrication. A 180-grit silicon carbide paper was used under running water to create a smear layer on the dentin surface.

**Pretreatment Protocols and GIC Bonding**

The prepared teeth were randomly divided into five experimental groups with three teeth each, based on the aggressiveness of the pretreatment protocol employed before the placement of a viscous restorative GIC (ChemFlex; Dentsply De Trey, Konstanz, Germany). The composition of the GIC and the conditioners are listed in Table 1. In all groups, includi-
Table 1 Glass-ionomer cements and conditioners used in this study

<table>
<thead>
<tr>
<th>GIC</th>
<th>Manufacturer</th>
<th>Batch number</th>
<th>Conditioner</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChemFlex</td>
<td>Dentsply DeTrey</td>
<td>9810000648</td>
<td>GIC liquid (10% polyacrylic acid)</td>
<td>Powder: Strontium alumino-fluorosilicate glass, Polyacrylic acid, Tartaric acid, Iron oxide and titanium oxide pigments</td>
</tr>
<tr>
<td></td>
<td>Konstanz, Germany</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ketac conditioner (25% polyacrylic acid)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ESPE, Seefeld, Germany</td>
<td>FW0046892</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bisco, Schaumburg, IL, USA</td>
<td>0000007171</td>
<td>Uni-Etch (32% phosphoric acid)</td>
<td></td>
</tr>
</tbody>
</table>

In the control specimens, the dentin surfaces were kept wet with water until they were blot dried. They were then left inverted on a piece of wet, lint-free tissue until the next treatment step.

Group C consisted of three teeth in which the GIC was applied without any pretreatment of the dentin surfaces. This served as the control group.

Group P teeth were pretreated with 10% PAA (ChemFlex liquid) for 10 s, and then bonded without further rinsing.

Group R teeth were pretreated with the ChemFlex liquid for 20 s. After rinsing the conditioner with distilled water for 10 s, each tooth was blot dried and left inverted on a piece of wet, lint-free tissue until ready for the placement of the GIC.

Group K teeth were pretreated with 25% PAA (Ketac Conditioner; ESPE, Seefeld, Germany) for 25 s. The dentin surfaces were then rinsed for 10 s, then treated as in group R.

Group H teeth were pretreated with 32% phosphoric acid (Uni-Etch; Bisco, Schaumburg, USA) for 15 s. After rinsing for 10 s, the dentin surfaces were treated as in group R.

The manufacturer's instructions recommend the use of a less viscous mix for the first increment and provide a green scoop that produces a powder:liquid ratio of 3.3:1. After applying a 0.5-mm-thick layer of this less viscous mixture to all specimens to enable better adaptation to the moist, etched dentin, the rest of the GIC buildup was made using the manufacturer's recommendation for a more condensable consistency, using their red scoop that provides a powder:liquid ratio of 3.8:1. All specimens were built up to a height of 5 mm. After the GICs set, all specimens were coated with Ketac-Glaze (ESPE) to protect the set material from the loss or gain of moisture. All teeth were then stored at 37°C, 100% humidity for 24 h before sectioning.

TEM Preparation

One tooth from each group was randomly selected for TEM examination. Two 1-mm-thick bonded dentin slabs were vertically sectioned from each tooth. They were fixed in Karnovsky's fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/L cacodylate buffer, pH 7.3) for 30 min and rinsed thoroughly with 0.1 mol/L sodium cacodylate buffer. The specimens were then post-fixed so that further elemental analyses could be done after TEM examination. The undemineralized slabs were then dehydrated in an ascending ethanol series (30% to 100%), immersed in propylene oxide as a transition fluid, and embedded in epoxy resin (TAAB 812 resin, TAAB Laboratories, Aldermaston, UK) at 60°C for 48 h.

The epoxy resin-embedded slabs from each
group were sectioned into 1 x 1 x 2 mm blocks that included the undemineralized dentin on one side and the GIC on the other. These blocks were then re-embedded in epoxy resin to facilitate subsequent preparation of thin sections. Seventy- to 90-nm-thick undemineralized sections were prepared with an ultramicrotome (Reichert Ultracut S, Leica, Vienna, Austria) using a diamond knife (Diatome, Biel, Switzerland), and collected on 200-mesh copper grids (TAAB laboratories, Aldermaston, UK). The unstained sections were examined using a transmission electron microscope (Philips EM208S, Eindhoven, The Netherlands) operating at 80 kV.

Microtensile Bond Testing

The other two specimens in each group were individually secured with sticky wax to a Plexiglass sectioning block. Using the nontrimming technique developed by Shono et al., beams with cross-sectional areas of approximately 0.81 mm$^2$ were prepared, with the GIC comprising the upper half of the beam and dentin comprising the lower half. Each tooth contributed equally, yielding between 20 to 23 beams for bond testing. Each beam was fixed to a modified Bencor Multi-T testing assembly (Danville Engineering, San Ramon, CA, USA) using cyanoacrylate (Zapit; DVA, Corona, CA, USA). The beams were pulled to failure under tension using a universal testing machine (Model 4440; Instron, Canton, MA, USA) at a crosshead speed of 1 mm per min. The exact dimensions of each fractured beam were then individually measured using a digital caliper (Model CD-6BS; Mitutoyo, Tokyo, Japan), from which the tensile bond strength was calculated. The failure modes of the bonds were initially evaluated at 30X with a stereomicroscope. Failures were classified as either adhesive between dentin and the GIC, cohesive failure within the GIC, or mixed (combinations of cohesive failure in the GIC and adhesive failure along the dentin surface).

One reason for using the microtensile bond test is to reduce the number of teeth required for investigations involving multiple variables, since human teeth are in short supply. Although only 3 teeth were bonded per group, and one was divided into 2 specimens for TEM examination, the other 2 teeth per group yielded 20 to 23 beams for bond testing that were pooled. As the sample size was small, we elected to use a very conservative nonparametric statistical analysis. Bond strength data obtained for the five experimental groups were analyzed with a nonparametric test (Kruskal-Wallis one-way ANOVA) using a statistical package (SigmaStat Version 2.03, SPSS, Chicago, IL, USA). Statistical significance was set in advance at the 0.05 level, while multiple comparisons were then performed using the Dunn’s post-hoc test.

Fractographic Analysis

The dentin sides of four randomly selected fractured beams in each group were examined using low temperature SEM, to minimize cracking of any residual GIC during specimen preparation for conventional SEM observation. The beams were kept at 100% humidity until the time of examination. They were secured with Zapit in holes that were pre-drilled in a copper stub. The entire stub was then plunged into liquid nitrogen for 2 min. After transferring to the low temperature preparation chamber (Cyroprep LT7400, Fisher Scientific, East Sussex, United Kingdom), the stub was maintained at -96°C for 30 min to allow sublimation of excess ice crystals from the fractured surfaces. After coating with gold-palladium, the temperature of the specimens was further reduced to -167°C. The stub was then inserted into the cryo-chamber of a scanning electron microscope (Cambridge Stereoscan 440, Cambridge, United Kingdom) and examined at 10 kV.

RESULTS

Ultrastructure of the GIC – Dentin Interfaces

In the absence of any surface pretreatment (group C), no obvious demineralization was observed on the surface of the intact intertubular dentin (Fig 1a). Smear layer remnants were embedded in a dark gray amorphous interphase that was well differentiated from the surrounding lighter gray polyalkenoate matrix of the GIC (Fig 1b). The intermediate layer of the GIC-dentin interface, in this specimen, resided completely within the smear layer.

In the specimen that was pretreated with 10% PAA for 10 s (group P), the dentin was partially demineralized to a depth of 0.5 μm to 0.8 μm (Fig 2a). A 0.3- to 0.5-μm-thick gray amorphous intermediate layer was present on top of this partially demineralized zone, and was demarcated from the GIC matrix.
Fig 1a A low-magnification TEM micrograph taken from an unstained, undemineralized section of the GIC-dentin interface in the control group C (no pretreatment). Smear layer remnants (S) are present along the surface of the undemineralized, intact dentin (U). Glass particles (G) are found within the polyalkenoate matrix (M) of the GIC. Electron-dense, circular bodies (black arrowheads) are also observed. They probably present incompletely reacted components of the GIC mixture. Separation of the GIC from the bonded substrate is evident along the interface (arrows). Bar = 500 nm.

Fig 1b A high-magnification unstained TEM micrograph from another location of the specimen shown in Fig 1a. The smear layer is present in the form of a thinner, more compact portion (between black arrowheads) that is about 1 μm thick on top of the undemineralized intact dentin (U), and a thicker superficial portion (arrow) that is very loosely dispersed within the polyalkenoate matrix (M) of the GIC. Electron-dense, needle-shaped and plate-like remnant apatite crystallites from the smear layer are embedded within a gray amorphous phase. We speculate that this phase represents the morphological manifestation of the interaction between calcium ions released by the partially demineralized apatites from the smear layer and the carboxyl groups of the polyalkenoic acid in the setting GIC. Together, they constitute the intermediate layer (IL) of the GIC-dentin interface, which in this control group resides completely within the smear layer. G: glass particle; U: undemineralized dentin. Bar = 200 nm.

(Fig 2b). These features were more prominent as the depth of demineralization increased (Fig 3a) in the specimen treated with 10% PAA for 20 s (group R). This resulted in the formation of a distinct interphase along the GIC-dentin interface. A higher magnification of the unstained, undemineralized section from this specimen (Fig 3b) that had not been exposed to osmium (which is commonly used during processing of specimens for electron microscopy) revealed the presence of collagen banding along the junction of the intermediate layer and the partially demineralized zone. They could be distinguished from the segregated, electron-dense remnant apatite crystallites that were also present within the partially demineralized collagen fibrils. The observation of collagen banding along the surface of the partially demineralized dentin was unusual, as the section was not positively stained.

Examination of the specimen pretreated with 25% PAA for 25 s (group K) showed that the depth of demineralization of the dentin increased to about 1.5 μm, with the surface collagen fibrils completely demineralized (Fig 4a). The position of the intermediate layer appeared to have shifted downward, and was located predominantly within the completely demineralized region of the intertubular dentin (Fig 4b). Faint collagen banding could also be recognized. However, there were also regions on top of the partially demineralized zone in which the collagen fibrils became “invisible.” These were areas where the unstained collagen fibrils were completely denuded of their apatite components. This diffusion gradient was even more noticeable in the specimen treated with 32% phosphoric acid (group H). A zone of completely demineralized collagen was created (Fig 5a) that was approximately 8 μm thick. More electron-dense regions with distinctive collagen banding could be observed around the
**Fig 2a** A low magnification unstained TEM micrograph of the GIC-dentin interface in group P (10% PAA 10 s, unrinsed). The smear layer is almost completely dissolved and the intact intertubular dentin (U) is partially dissolved by the polyacrylic acid, resulting in a partially demineralized zone that is 0.8 µm thick. A circumferential layer of peritubular dentin (Pd) is present around the dentinal tubule, in which smear plug remnants are present (arrowhead). M: polyalkenoate matrix of the GIC. Bar = 1 µm.

**Fig 2b** A high-magnification unstained TEM micrograph of an area similar to that shown in Fig 2a. Remnant apatite crystallites are found within the zone of partially demineralized dentin (P). On top of this zone is a 200-nm-thick, gray intermediate layer (IL) that can be distinguished from the overlying GIC matrix (M). We speculate that this phase, similar to that in Fig 1b, represents the morphological manifestation of the interaction of calcium ions from the partially demineralized dentin with the PAA. It is possible that this phase also extends into the thin layer of partially demineralized dentin that is created by PAA pretreatment. Electron-dense structures (arrowheads) within this intermediate layer are probably remnants of the smear layer, as they are not observed in the groups that were conditioned with more aggressive protocols. Strontium aluminofluorosilicate glass particles can be identified within the GIC matrix. A large, typical glass particle consists of a central electron-dense glass core (C) and a peripheral siliceous hydrogel layer (H). U: undemineralized intertubular dentin. Bar = 300 nm.

**Fig 3a** A low-magnification unstained TEM micrograph of the GIC-dentin interface in group R (10% PAA 20 s, rinsed). A 500 nm thick gray, amorphous intermediate layer (IL) is well demarcated from the GIC matrix (M), and is located well above the 1.2-µm-thick partially demineralized zone (P). U: undemineralized intertubular dentin; T: dentinal tubule. Bar = 300 nm.

**Fig 3b** A higher magnification of Fig 3a. Electron-dense apatite crystallites are readily identified within the partially demineralized zone (P). Along the junction of this zone and the intermediate layer (IL), banded collagen fibrils (arrowhead) can also be seen. The observation of banding is unusual, as the section was not positively stained (see also Fig 5b). M: GIC matrix; U: undemineralized intertubular dentin. Bar = 300 nm.
Fig 4a A low-magnification unstained TEM micrograph of the GIC-dentin interface in group K (25% PAA 25 s, rinsed). The more aggressive pretreatment protocol results in a 1.5-μm-thick demineralized zone, the superficial portion of which is completely demineralized (C) and the basal portion partially demineralized (P). Silhouettes of upright-positioned collagen fibrils can be identified along the dentin surface (arrowhead). Dentrinal tubules are rendered patent and contain glass particles around the tubular orifices (arrow) as well as the polyalkenoate matrix (M) within the tubules. Pd: undemineralized peritubular dentin; U: undemineralized intertubular dentin. Bar = 300 nm.

Fig 4b A high-magnification TEM micrograph of an area similar to that depicted in Fig 4a. A gray, amorphous intermediate layer (IL) can be identified mostly within the completely demineralized portion of the demineralized zone. Similar to Fig 3b, faintly banded collagen fibrils can be seen within this layer (arrowhead). In contrast, remnant apatite crystallites within the underlying partially demineralized zone (P) are segregated and more electron-dense. As the depth of demineralization is deeper than the previous groups, ion exchange between the setting GIC and the partially demineralized collagen fibrils could result in the formation of an intermediate layer that resides predominantly within the superficial layer of completely demineralized collagen instead of along the dentin surface. We speculate that the electron-lucent region in this unstained section indicated by the asterisk probably represents collagen fibrils that are completely denuded of apatite crystallites, but are too deep to be infiltrated by the PAA from the setting GIC, so that no interaction products were deposited within the collagen fibrils and/or interfibrillar spaces. M: GIC matrix; U: undemineralized intertubular dentin. Bar = 300 nm.

Fig 5a A low-magnification unstained TEM micrograph of the GIC-dentin interface in group H (32% phosphoric acid 15 s, rinsed). This aggressive conditioning protocol produced a completely demineralized zone (C) that was 8 μm thick. Dentrinal tubules (T) were rendered completely patent, but are not clearly demonstrated in this micrograph due to the cutting angular. A gradient of grayness can be identified, being most noticeable along the dentin surface and the lateral walls of the dentrinal tubule (arrows). These regions are the first to be demineralized by the penetrating phosphoric acid conditioner, and the collagen fibrils should thus be completely devoid of hydroxyapatite crystallites. However, distinctive banding can be discerned within the collagen fibrils at higher magnification (Fig 5b). The gray regions probably represent the intermediate layer (IL) that is formed between the ions from the GIC fillers dissolved in the infiltrating PAA reacting with the demineralized dentin matrix. There is probably a limiting size for polyelectrolytes with high molecular weights that restricts how far they can diffuse through the bed of demineralized collagen. Incomplete infiltration of the PAA probably results in the formation of a 1- to 1.5-μm-thick intermediate layer within the superficial part of the completely demineralized dentin. M: GIC matrix; U: undemineralized intertubular dentin. Bar = 1 μm.

Fig 5b A high-magnification TEM micrograph of an area similar to that depicted in Fig 5a. A gray, amorphous intermediate layer (IL) can be identified mostly within the completely demineralized portion of the demineralized zone. Similar to Fig 3b, faintly banded collagen fibrils can be seen within this layer (arrowhead). In contrast, remnant apatite crystallites within the underlying partially demineralized zone (P) are segregated and more electron-dense. As the depth of demineralization is deeper than the previous groups, ion exchange between the setting GIC and the partially demineralized collagen fibrils could result in the formation of an intermediate layer that resides predominantly within the superficial layer of completely demineralized collagen instead of along the dentin surface. We speculate that the electron-lucent region in this unstained section indicated by the asterisk probably represents collagen fibrils that are completely denuded of apatite crystallites, but are too deep to be infiltrated by the PAA from the setting GIC, so that no interaction products were deposited within the collagen fibrils and/or interfibrillar spaces. M: GIC matrix; U: undemineralized intertubular dentin. Bar = 300 nm.
Fig 5b A higher magnification of another section after demineralization and staining with lead citrate and uranyl acetate that react with the PAA to form electron-dense deposits. This demonstrates that the PAA does not diffuse into the demineralized region more than 1 to 2 μm. Bar = 1 μm.

Fig 5c A higher magnification of Fig 5a illustrating the unusual banding patterns (arrowheads) that are present within some completely demineralized, unstained collagen fibrils (C) along the dentin surface. Interfibrillar spaces are also infiltrated by a material of similar electron density. M: GIC matrix. Bar = 300 nm.

Fig 6a An unstained, undemineralized micrograph showing the presence of “seeds” (arrowheads) within the hydrogel layer (H) of a strontium aluminofluorosilicate glass particle. Each electron-lucent “seed” has an electron-dense periphery that is connected via pinwheel-like, electron-dense extensions to the center. They vary substantially in size from particle to particle. However, they are of a similar size range within a single glass particle. The composition and function of these “seeds” are currently unknown. C: glass core; M: polyalkenoate matrix of the GIC. Bar = 300 nm.

Fig 6b Another unstained section showing the presence of much smaller “seeds” within a glass particle. The roughly parallel, electron-lucent spaces (arrowhead) are chatters created in the section that are caused by cutting of the diamond knife through a brittle material. C: glass core; H: siliceous hydrogel layer; M: polyalkenoate matrix of the GIC. Bar = 500 nm.

dentin surfaces and the lateral walls of the dentinal tubules. These regions were the first to be demineralized by the penetrating phosphoric acid conditioner, and the collagen fibrils were completely devoid of apatite crystallites. Incomplete infiltration of the PAA resulted in the formation of a 1- to 1.5-μm-thick intermediate layer within the superficial part of the completely demineralized dentin. This can be seen in a demineralized, stained section (Fig 5b). A higher magnification of the collagen
**Table 2** Microtensile bond strength (µTBS) of the high-strength glass-ionomer cement bonded to sound dentin using various pretreatment protocols

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of beams tested</th>
<th>µTBS (MPa) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>20 (1)</td>
<td>7.2 ± 1.7 a</td>
</tr>
<tr>
<td>P</td>
<td>23 (0)</td>
<td>14.0 ± 3.7 b</td>
</tr>
<tr>
<td>R</td>
<td>22 (0)</td>
<td>14.0 ± 3.4 b</td>
</tr>
<tr>
<td>K</td>
<td>21 (0)</td>
<td>15.0 ± 2.4 b</td>
</tr>
<tr>
<td>H</td>
<td>22 (0)</td>
<td>15.3 ± 3.2 b</td>
</tr>
</tbody>
</table>

* Values are means ± SD. Groups identified with the same superscripts are not significantly different (p < 0.05). The values in parentheses indicate the number of specimens that failed during preparation before they could be tested.

**Group designations**: C: control - no pretreatment; P: 10% polyacrylic acid (PAA) 10 s, no rinsing; R: 10% PAA 20 s, rinsed; K: 25% PAA 25 s, rinsed; H: 32% phosphoric acid, 15 s, rinsed.

bonding along the dentin surface from an unstained, undemineralized section is shown in Fig 5c.

Within the polyalkenoate matrix of the GIC, surface-reacted glass particles were readily identified. They possessed an electron-dense core and a peripheral electron-lucent, siliceous hydrogel layer (Fig 6a). Numerous "seeds" were consistently observed within the hydrogel of these glass particles. The "seeds" varied substantially in size from glass particle to glass particle (Fig 6b). However, they were of a similar size range within the same glass particle.

**Microtensile Bond Strengths and Fractographic Analysis**

Mean microtensile bond strength (µTBS) for the five experimental groups are shown in Table 2. The bond testing revealed that the mean µTBS for each of the four groups varied from 7.2 to 15.3 MPa. One-way ANOVA based on ranks (Kruskal-Wallis) indicated a significant difference among the groups tested. The Dunn's multiple comparison test showed that the four GIC groups bonded with dentin pretreatment were not significantly different from one another in terms of mean bond strength. They were, however, significantly higher (p < 0.05) than the control group, in which the GIC was bonded to smear layer-covered dentin.

SEM examination of the fractured interfaces showed that adhesive failure was the predominant failure mode in group C (Fig 7a). A partially peeled-off layer along the dentin side of a fractured surface (Fig 7b) corresponded with the intermediate layer that incorporated the smear remnants in the TEM observation. In contrast, mixed failure was the predominant failure mode in the other four groups (Fig 7c). At higher magnification, an intermediate layer was discernable between the surface of the intact dentin and the fractured GIC in groups P and R (Fig 7d). The intermediate layer that was shown by TEM to be located within the demineralized dentin could not be seen in groups K and H, since SEM observations only reveal surface details. Patent dentinal tubules were identified from the surface of the fractured dentin (Fig 7e). Figure 8 summarizes the results in a schematic form.

**DISCUSSION**

There are very few TEM studies of GICs or glass ionomer-based adhesives available in the literature. In particular, ultrastructural reports of the interaction between chemically cured GIC and dentin are lacking due to the difficulty involved in the preparation of GIC-dentin interface for TEM examination. Unlike resin-dentin interfaces, the polyalkenoate matrices of GICs are soluble in acidic buffers or chelating agents and are rapidly dissolved during laboratory demineralization of the specimens. Our pilot TEM investigation of these five experimental groups using stained, demineralized sections revealed severe dissolution of the matrices. Moreover, the glass particles were also dissolved and became empty shells that were
Fig 7a  A low-magnification SEM micrograph of the dentin side of a representative fractured beam from control group C (no pretreatment) that was stressed to failure using the microtensile bond test. The failure mode is adhesive in nature. At this magnification, a thin, partially peeled-off layer (pointer) can be discerned on the fractured surface, leaving the dentin (D) exposed in some areas. Bar = 20 µm.

Fig 7b  A higher magnification of Fig 7a showing that the partially peeled-off layer (arrows) corresponds with the TEM appearance of an intermediate layer (IL) that resides completely within the smear layer. D: intact dentin. Bar = 10 µm.

Fig 7c  A low-magnification SEM micrograph of the dentin side of a fractured beam from group R (10% PAA 20 s, rinsed). A mixed failure mode can be seen, with a combination of adhesive failure along the surface of the intact dentin (D) and cohesive failure within the GIC (G). This mixed failure mode is also representative to groups P, K and H. Some voids (V) can be discerned within the fractured GIC. Cracks are present on the surface of the exposed GIC even when examined using cryo-SEM. They are probably caused by dehydration of the thin layer of fractured GIC when the fractured beam was examined with a stereomicroscope to determine the failure mode. IL: intermediate layer. Bar = 20 µm.

Fig 7d  A higher magnification of Fig 7c, showing the presence of an intermediate layer (IL) between the fractured GIC (G) and intact dentin (D). Bar = 10 µm.
Fig 7e  A SEM micrograph showing a higher magnification of a representative mixed failure mode in group H (32% phosphoric acid 15 s, rinsed). Exposed dentinal tubules (pointers) are readily discerned from the surface of the intact dentin (D). Isolated islands of fractured GIC (G) can also be identified. An intermediate layer cannot be recognized on top of the intact dentin. Based on the previous TEM observations, the intermediate layer is probably represented by the exposed surface of the intact dentin. Bar = 10 μm.

Fig 8  Schematic summarizing the depth of demineralization of dentin by the various conditioners and the depth of the intermediate layer within the underlying dentin.

surrounded by artifactual surface precipitation of electron-dense amorphous deposits (Tay, unpublished results). There is further evidence that unslisanized fluoroaluminosilicate glass particles, when used as fillers for experimental composites, are completely dissolved by the uranyl acetate (pH 4.2) used for staining of undemineralized TEM sections (Tay, unpublished results). Therefore, we elected to report the ultrastructure of the GIC-dentin interfaces using unstained, undemineralized sections. These sections are much more difficult to prepare than undemineralized dentin bonded using resin-modified GICs or resin-based adhesives. This is because of the lack of resin support and the difficulty of the hydrophobic epoxy resin to infiltrate the hydrophilic polyalkenoate matrix of the GIC.

TEM examination of undemineralized, unstained specimens of GIC bonded to dentin has expanded our understanding of the structure of GIC as well as its interaction with the bonding substrate. The presence of “seeds” within the hydrogel layer of the glass particles in GIC has been previously reported but not discussed. They are not unique to ChemFlex, as similar “seeds” are also in the fluoroaluminosilicate glass particles of a glass-ionomer based, all-in-one dentin adhesive known as Reactomer Bond (Shofu, Kyoto, Japan; Tay et al, unpublished results). Wilson’s group explained that G-200
glass in the glass-ionomer cement has two phases: a continuous calcium aluminosilicate matrix and partly crystalline calcium fluoride-rich droplets, the nature of which depends on the thermal history of the glass. The setting process of the cement takes place when the glass is mixed with polyacrylic acid. It has two overlapping reaction stages corresponding to a rapid leaching of calcium ions from the noncrystalline portion of the droplets, followed by a slower release of aluminum (and some calcium) from the major glass phase. These processes are affected by the microstructure and microcomposition of the glass.

Our TEM results also confirmed the existence of an interphase along the GIC-dentin interface, as reported by previous SEM studies. This intermediate layer was shown in a recent spectroscopic study to contain elements from the bidirectional diffusion of ions between the GIC and dentin. In the present study, the intermediate layer could be located either within the smear layer, on the surface of the partially demineralized dentin, or even within the superficial part of the completely demineralized dentin (ie, around the interfibrillar spaces, Fig 8). The inclusion of either smear layer remnants or banded collagen fibrils within the intermediate layer may be explained by the aggressiveness of different pretreatment protocols in removing the smear layer and demineralizing the underlying intact dentin. We speculate that the unusual observation of banded collagen in undemineralized sections (Fig 5c) may be caused by the interaction of free metallic ions (Ca\(^{2+}\), Sr\(^{2+}\) or Al\(^{3+}\)) in the demineralized collagen network and the PAA that may adhere chemically to either remnant hydroxyapatite crystallites or collagen. Similar to what may occur around the interfibrillar spaces, deposition of amorphous, insoluble, organic salts within the gap zones of the collagen molecules could result in the formation of vague banding within the unstained collagen fibrils.

In the absence of any pretreatment, the intermediate layer incorporated only smear remnants, and no demineralization was observed in the underlying intact dentin (Fig 8). This weak link between the smear layer and the underlying dentin substrate was reflected by the significantly lower bond strength in the control group, and the partial separation of the intermediate layer from the underlying dentin that was depicted in the fractographic analysis. Peutzfeldt and Asmussen reported that PAA pretreatment improves the bond of GIC to rough dentin surfaces with thicker smear layers, but not for smoother dentin surfaces with thinner smear layers. In the present study, dentin was abraded with 180-grit SiC paper, which produces a 0.7- to 3.3-μm-thick smear layer. There is the possibility that the intermediate layer may not completely incorporate the smear remnants in areas where the smear layer is thick. This would be comparable to the weakness observed in early generation dentin adhesives that attempted to bond only to smear layers, but without demineralizing and infiltrating the underlying dentin to form hybrid layers.

Wesenberg et al reported the presence of a partially demineralized subsurface zone beneath an outer zone of increased calcium and phosphorus content in unconditioned, GIC-bonded dentin. As the PAA in the GIC mixture exhibits some intrinsic acidity prior to setting, the difference between the observations in our control group and the results of Wesenberg et al may be due to the different smear layer thicknesses in the two studies as well as differences in the concentration of the PAA. Nevertheless, in the other four groups that were pretreated with either PAA or phosphoric acid, we observed the coexistence of partially or completely demineralized subsurface zones beneath the intermediate layers. The variation in the thickness of these subsurface demineralized zones must necessarily be related to the aggressiveness of the pretreatment protocols. There also appeared to be a downward migration (Fig 8) of the intermediate layers from the surface of the partially demineralized dentin in the less aggressive protocols (ie, groups P and R) to within the superficial portions of the completely demineralized dentin in the more aggressive protocols (ie, groups K and H). Such a relationship may be associated with the limited diffusivity of the PAA and the availability of calcium ions within different depths of the demineralized dentin. It is also notable that group P was the only group in which there was the likelihood that the intermediate layer corresponded with the depth of demineralization.

Intermediate layers of GIC that incorporated intact, banded collagen fibrils may be comparable to the interdiffusion zones (hybrid layers) in acid-conditioned, resin-infiltrated dentin. Although their mechanisms of formation are different, both the PAA and resins have to diffuse downward through a bed of demineralized collagen fibrils in order to infiltrate the interfibrillar spaces for either chemical adhesion or micromechanical retention. This diffusion gradient was readily apparent in GIC bonded to phosphoric acid-conditioned dentin. Unlike treat-
ment with 25% PAA, in which some of the PAA may be retained by chemical bonding to the partially demineralized hydroxyapatites and/or collagen after rinsing, the demineralized collagen in the phosphoric acid group must rely on the infiltration of PAA from the setting cement. We did not anticipate the presence of an intermediate layer that contains bonded collagen in phosphoric acid-etched dentin, as the apatite crystallites are completely dissolved within the demineralized collagen network. However, there may still be residual calcium ions within the interfibrillar spaces of this network even after rinsing. The metals may also have originated from the action of PAA on the filler particles. We are currently investigating the relation between intermediate layers and partially/completely demineralized dentin in the various GIC-dentin interfaces with the use of SEM/energy dispersive x-ray (STEM/EDX).

Previous studies of dentin adhesives that contain polyalkenoic acid copolymer showed that this component was preferentially retained on the acid-etched dentin surface, with minimal diffusion (ca 500 nm) into the underlying demineralized collagen. This may be due to the low permeability of this high molecular weight resin-modified component. Unlike the diffusion of dentin adhesive components, such as hydroxyethyl methacrylate, into the interfibrillar spaces, there is a possibility that these spaces may not be completely infiltrated by high molecular weight PAA in chemically cured (ie, acid-base reaction) GICs. The situation may even be worsened by the collapse of the demineralized collagen network when acid-etched dentin is desiccated before GIC placement. Based on our ultrastructural observations, we initially speculated that there may be a deterioration of the GIC-dentin bond with the use of more aggressive pretreatment protocols that leave a bed of denuded collagen within the subsurface demineralized dentin. Our microtensile bond strength data, however, did not support such a speculation. As there was no significant difference in the four pretreated groups, we have to accept the null hypothesis that ultrastructure of the GIC interfaces do not correlate with their respective microtensile bond strength.

Although the version of microtensile bond test employed in this study was discriminative enough to distinguish the unconditioned control group from the pretreated groups, it did not allow isolation of any difference in the latter four groups. When the fracture modes were further analyzed, most failures were mixed failures and cohesive failures in the GIC in these four groups. Thus, the similar bond strengths in these groups (14.0 to 15.3 MPa) represent the cohesive strength of the GIC tested rather than its true adhesive strength to dentin. In the two aggressively pretreated groups (K and H), it is possible that the GIC is bonding to denuded collagen via the intermediate layer. If this hypothesis is correct, then the absence of cohesive failure within the demineralized zones suggests that the adhesive strength of the GIC to dentin is below the ultimate tensile strength of demineralized dentin collagen, which was reported by Sano et al to be in the range of 30 MPa.

It may also be concluded that bonding of Chem-Flex GIC to dentin may be effectively achieved with the use of mild dentin conditioning protocols such as the 10% PAA. The use of more aggressive conditioning protocols that demineralize dentin to a depth beyond that necessary for chemical interaction via the formation of the intermediate layer does not enhance the dentin-GIC restorative bond strength. One of the potential disadvantages of a more aggressive acid pretreatment protocol is that removal of the smear plugs may increase the permeability of dentin and outward flux of dentinal fluids during bonding. This may further dilute the chemically cured GICs, and produces weaker bonds to dentin that are similar to the effects of dentin perfusion on the bonding of resin-modified GICs. Another potential disadvantage is the presence of denuded collagen beneath GIC-bonded dentin, which has to be further investigated and confirmed. However, even if this situation exists, it may not be easily detected clinically if both the cohesive strength of the GIC and the bond of the GIC to dentin are weaker than the ultimate tensile strength of demineralized collagen. Only when collagen fibrils can be seen on the GIC side of a failed bond could one interpret the failure as being due to degradation of the naked collagen fibrils. Although some clinicians advocate the total-etch technique for placing conventional GICs, we are concerned that this technique etches dentin deeper than the intermediate layer can form, making the durability of such restorations questionable. Clearly, the interphase between dentin and ionomers must be investigated further using other glass-ionomer cements and related materials.
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REFERENCES


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