Influence of Heliobond on Immediate Nanoleakage and Microtensile at Resin-Dentin Interface (An In Vitro Study)

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Abstract

Purpose: The purpose of the present study is to evaluate nanoleakage and microtensile bond strength of different resin composite materials bonded to dentin.

Material and methods: Teeth will be divided into three main equal groups according to filling material. Each main equal group will be subdivided into two equal subgroups according to application mode (self-etch vs. Etch and rinse mode) (n=20). Then each subgroup will be further divided in to smaller Subgroups. Subgroup 1: using filtek TM Z250 with G-bond plus (G-aenial) (self-etching mode), then application of hydrophopic resin coating (Heliobond). Subgroup 2: using filtek TM Z250 with G-bond plus (G-aenial) (self-etching mode), without application of hydrophobic resin coating (Helio bond). Subgroup 3: using filtek TM Z250 with G-bond plus (G-aenial) (etch and rinse mode), then application of hydrophobic resin coating (Heliobond). Subgroup 4: using filtek TM Z250 with G-bond plus (G-aenial) (etch and rinse mode), without application of hydrophobic resin coating (Heliobond).

Conclusion: An extra hydrophobic layer coating improved the immediate in vitro performance (µTBS and NL) of the universal adhesive systems that studied in SE mode. However, NL pattern is material-dependent and aging stability seems not to be related with the adhesive strategy.

Keywords: SEM, EDX, microtensile, nanoleakage, Heliobond, G-aenial bond


1. Introduction

The tensile bond strength of an adhesive system is mostly influenced by the hybrid layer, followed by resin tags in the dentinal tubule and finally by chemical bonding [1,2]. However, porosities can be observed at the bottom of hybrid layers. This phenomenon was termed “nanoleakage” and was first described in 1994 by Sano et al. [3], they noticed incomplete infiltration of hybrid layers following acid-etching during adhesive bonding techniques [3]. Silver nitrate was originally used to detect microleakage around composite restorations [4]. The low molecular weight silver nitrate tracer diffusion is similar to that of water and has been used to trace nanometer-sized water-filled spaces or “nanoleakage” within bonded interfaces, examined under scanning or transmission electron microscopy [5,6].

Several factors influence the development of nanoleakage, including the type of solvent (water vs. acetone) [7,8,9,10], the individual chemical constituents system (e.g. hydroxyethyl methacrylate (HEMA), and bisphenol glycolal methacrylate (Bis-GMA)), and the different molecular weights of constituents ranging from 130 (HEMA) to 513 (Bis-GMA) as well as other additives (among others maleine acid or glutaraldehyde) [5]. In addition, and the mode of application when using these materials (moist bonding vs. dry bonding), as dry bonding of etch and rinse adhesives increases the occurrence of nanoleakage [5]. This phenomenon is explained by the collapse of acid-etched dentin matrix that interferes with resin infiltration. The nanoleakage effect has been discussed to be one factor negatively affecting the quality of dentinal bonding [10,11,12].

Recently, developed resin adhesives contain more acidic hydrophilic monomers, and higher amounts of water to improve monomer impregnation into wet dentin substrate, resulting in lower degrees of polymerization of adhesive resin. This results in increased silver uptake into the hybrid and adhesive layers (i.e. increased nanoleakage).

Influence of nanoleakage on microtensile bond strength was determined using different visualization techniques [5]. For instance, resin–dentin specimens were prepared and immersed in silver nitrate that penetrated into nanoleakage. Subsequently, specimens were broken at the adhesive interface (commonly using tensile testing or shear bond strength testing procedures), and nanoleakage was visualized indirectly on the exposed surface using scanning electron microscopy (SEM) [6].
It has been postulated that, between polymerization shrinkage and microtensile bond strengths (μTBS) a highly significant correlation was found because forces developed during the polymerization of dental restorative composites placed in a restricted setting, cause tension in the material, with possible subsequent distortion of the bond to the tooth [13]. Furthermore, the quality of the bond between tooth and restorative materials could, also, be affected by the incompatibility between adhesive and restorative material as well as by the surface tensions of the two components coming into contact with each other [14].

The extent of this shrinkage influences the tension state generated at the interface composite/dental structure and, commonly, compromises the bond integrity at this region. In addition, the polymerization shrinkage of composites is also influenced by the geometric form of the cavity. When the ratio between the bounded to unbounded surfaces is higher than two, the stress generated by the composite shrinkage may exceed the bond strength to the cavity walls and produce marginal gaps [15]. When these problems are added to an incorrect placement technique and finishing mistakes, marginal leakage, inadequate anatomic form and proximal contacts occur clinically, which lead to a consequent reduction in the longevity of the restoration [16,17].

2. Materials and Methods

2.1. Three Types of Adhesive Systems (self-etch or etch and rinse mode):

Filtek TM Z250, (Figure 1) Scotchbond Universal Adhesive also known as Single Bond Universal (Figure 2). G-bond plus (G-aenial bond); (Figure 3). Hydrophobic resin coating; with or with-out, Heliobond (Figure 4).

<table>
<thead>
<tr>
<th>Product name</th>
<th>Composition</th>
<th>manufacturer</th>
<th>Batch number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtek™ Z250 shade A1</td>
<td>Bis-GMA, Bis-EMA, TEGDMA, UDMA, zirconia, silica (82 wt%/60 vol%)</td>
<td>3M ESPE, USA</td>
<td>N636467</td>
</tr>
<tr>
<td>Single Bond Universal</td>
<td>1-Etchant: 32% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminum oxide (Scotchbond Universal Etchant)</td>
<td>SBU, 3M ESPE, St. Paul, MN, USA</td>
<td>N601958</td>
</tr>
<tr>
<td>G-BOND plus (G-aenial)</td>
<td>Acetone, dimethacrylate, 4 methacryloyloxymethyltrimellitate anhydride, phosphoric acid ester monomer, silicon dioxide, photo initiator, distilled water</td>
<td>GC Corporation Tokyo, Japan</td>
<td>N1510051</td>
</tr>
<tr>
<td>Heliobond</td>
<td>bis-GMA, TEGDMA, initiators, stabilizers</td>
<td>Ivoclar Vivadent, Schaan, Liechtenstein</td>
<td>U34134</td>
</tr>
</tbody>
</table>

2.2. Specimen Preparation

A flat occlusal dentin surface was exposed in all teeth after wet-grinding the occlusal enamel with a slow-speed water-cooled diamond disk Isomet, Buehler Ltd., Lake Bluff, IL, USA. The exposed dentin surfaces were further polished with wet #600-grit silicon-carbide paper for 60 s to standardize the smear layer [20]. The adhesive systems were applied according to the respective manufacturer’s instructions, except for G-bond Plus, for which the manufacturer does not recommend dentin etching with phosphoric acid. Furthermore, the respective manufacturers do not recommend the application of Heliobond. Composite resin crowns were built up in two increments of 2 mm each. Except for bulk fill composite applied in one increment. Each increment was light-cured for 40 s using a LED light-curing unit set at 1200 mW/cm² (Radical, SDI Limited, Bays water, Victoria, Australia) (Figure 6).
the specimens were sectioned longitudinally in mesio-distal and bucco-lingual directions across the bonded interface with a slow-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) (Figure 7) to obtain resin-dentin beams (Figure 9) with a cross sectional area of approximately 0.8 mm² measured with a digital caliper (Digimatic Caliper, Mitutoyo, Tokyo, Japan). (Figure 8) half of beams used for microtensile bond strength test and the other beams used for nanoleakage evaluation.

2.3. Adhesive and Resin Composite Application

Regarding subgroup 1: (self-etching mode) using G-bond plus (G-aenial): Apply using a micro brush. Leave undisturbed for 10s after the end of application. Dry thoroughly for 5s with oil free air under maximum air pressure. Use vacuum suction to prevent splatter of the adhesive. Light cure for 10s. Then apply a very thin layer of Heliobond with a micro brush on the dental surface. Apply air blower to achieve an optimally thin layer. Light-cure for 10s. Filetek Z250 composite resin crowns were built up in two increments of 2 mm each. Each increment was light-cured for 40s using a LED light-curing unit (Figure 6).

Regarding subgroup 2: the same technique used in subgroup 1 with one exception Heliobond will not applied.

Regarding subgroup 3: (etch and rinse mode) using the same etching gel(Figure 5) used in subgroup 3, then application of G-bond plus (G-aenial), application of Heliobond adhesive as mentioned in subgroup 2.

Regarding subgroup 4: the same technique used in subgroup 3 with one exception Heliobond will not applied.

2.4. Testing Procedures

For microtensile bond strength (µTBS): the resin-dentin bonded beam was attached to resin-dentin bonded beam holder (Figure 10) with tetric-flow flowable composite (3M adhesive) and tested under tension (Model5565, Instron Co., Canton, MA, USA) (Figure 11) at 0.5 mm/min until failure. The µTBS values were calculated by dividing the load at failure by the cross-sectional bonding area [18,19]. The µTBS values (MPa) of all beams from the same tooth were averaged for statistical purposes.
**For nanoleakage analysis:** the beams were placed in an ammonical silver nitrate solution in darkness for 24 h [21], rinsed thoroughly in distilled water, and immersed in photo developing solution for 8 h under a fluorescent light to reduce silver ions into metallic silver grains. Specimens were polished with wet 600 grit Sic paper. Resin–dentin interface were analyzed with a scanning electron microscope (Philips, XL 30, Eindhoven, The Netherlands) (Figure 12), also analyzed using Energy dispersive X-ray spectrometry (EDX Philips, XL 30 W/TMP, Eindhoven, The Netherlands) (Figure 12). The micrograph was taken in the center of the beam [22]. The mean NL (%) of all beams from the same tooth was averaged for statistical purposes. Comparison between the twelve different subgroups was made using four-dimensional mapping which was performed over 100 mm x 100 mm areas across the resin-dentine bonded interface, these areas covered the adhesive layer. The HL (hybrid), partially demineralized and un-affected dentine was visualized and focused at 1000 x magnification. Amount of silver grains that was penetrated at resin-dentin interface was calculated and statistically analyzed through energy levels of EDX analysis [23].

3. Results

3.1. μ-Tensile Bond Strength (μ-TBS)

**Self-etching application mode:**

Table 1: Comparison of μ-tensile bond strength results (Mean±SD) between all composite groups as function of adhesive type, resin coating with self-etching application mode. Figure 12: Column chart of μ-tensile bond strength mean values for all composite groups as function of adhesive type, resin coating with self-etching application mode.

<table>
<thead>
<tr>
<th>Application mode (D)</th>
<th>Self-etching (D1)</th>
<th>t-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin coating (C)</td>
<td>With (C1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filtek™ Z250 (A3)</td>
<td>27.2±2.1</td>
<td></td>
<td>0.0001*</td>
</tr>
<tr>
<td>ANOVA</td>
<td>P value</td>
<td>0.0004*</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

Different letter in the same column indicating statistically significant difference (p < 0.05). *: significant (p < 0.05), ns: non-significant (p>0.05)

**Etch and rinse application mode**

Table 2: Comparison of μ-tensile bond strength results (Mean±SD) between all composite groups as function of adhesive type, application mode and resin coating. Figure 13: Column chart of μ-tensile bond strength mean values for all composite groups as function of adhesive type, resin coating with etch-&-rinse application mode

<table>
<thead>
<tr>
<th>Application mode (D)</th>
<th>Etch and rinse (D2)</th>
<th>t-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin coating (C)</td>
<td>With (C1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filtek™ Z250 (A3)</td>
<td>21.3±2.7</td>
<td></td>
<td>0.0317*</td>
</tr>
<tr>
<td>ANOVA</td>
<td>P value</td>
<td>0.0002*</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

Different letter in the same column indicating statistically significant difference (p < 0.05). *: significant (p < 0.05), ns: non-significant (p>0.05)

**Interaction between variables**

Self-etching application mode with Resin coating; it was found that Filtek™ Z250 with heliobond recorded the high statistically significant (P≥0.05) μ-tensile bond strength mean value as indicated by one way ANOVA followed by pair-wise Tukey’s post-hoc tests.

without Resin coating; it was found that Filtek™ Z250 without heliobond recorded the lower statistically significant (P<0.05) μ-tensile bond strength mean value as indicated by one way ANOVA followed by pair-wise Tukey’s post-hoc tests.

Resin coat vs. non-coated: it was found that group with Resin coating recorded statistically significant (P<0.05) higher μ-tensile bond strength mean value than groups without Resin coating as indicated by paired t-test

**3.2. For Nanoleakage**

Nanoleakage results (%) for all composite groups as function of adhesive type, application mode and resin coating.
coating are summarized in Table 3. Figure 15: Column chart of total nanoleakage mean values as function of application mode. Figure 16: Column chart of total nanoleakage mean values as function of resin coat application.

<table>
<thead>
<tr>
<th>Resin composite filling (A)</th>
<th>Filtek™ Z250 (A3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesive (B)</td>
<td>G-bond plus (B2)</td>
</tr>
<tr>
<td>Resin coating (C)</td>
<td>With (C1)</td>
</tr>
<tr>
<td>Application mode (D)</td>
<td>Self-etching (D1)</td>
</tr>
<tr>
<td></td>
<td>Etch and rinse (D2)</td>
</tr>
</tbody>
</table>

**Table 3**

**Figure 15.**

**Figure 16.**

**Regarding subgroup 1:**

Figure 17a: Backscattered electron image of SEM (a) and corresponding EDX spectrum (b) of the fractured surface of resin dentin beam side at a magnification 1000 x. Figure 17b: Element profile for Filtek™ Z 250 + G-Bond plus (self-etch mode) with hydrophobic resin coating.

Table 4: Amount of silver at energy level (L) in Figure 17a.

<table>
<thead>
<tr>
<th>Element</th>
<th>Wt %</th>
<th>At %</th>
</tr>
</thead>
<tbody>
<tr>
<td>O K</td>
<td>71.40</td>
<td>86.03</td>
</tr>
<tr>
<td>P K</td>
<td>16.59</td>
<td>10.32</td>
</tr>
<tr>
<td>CIK</td>
<td>02.57</td>
<td>01.40</td>
</tr>
<tr>
<td>AgL</td>
<td>07.59</td>
<td>01.36</td>
</tr>
<tr>
<td>CaK</td>
<td>01.85</td>
<td>00.89</td>
</tr>
</tbody>
</table>

**Table 4**

**Figure 17a.**

**Figure 17b.**

**Regarding subgroup 2:**

Figure 18a: Backscattered electron image of SEM (a) and corresponding EDX spectrum (b) of the fractured surface of resin dentin beam side at a magnification 1000 x. Figure 18b: Element profile for Filtek™ Z 250 + G-Bond plus (self-etch mode) without hydrophobic resin coating.

Table 5: Amount of silver at energy level (L) in Figure 18a.

<table>
<thead>
<tr>
<th>Element</th>
<th>Wt %</th>
<th>At %</th>
</tr>
</thead>
<tbody>
<tr>
<td>O K</td>
<td>12.22</td>
<td>36.80</td>
</tr>
<tr>
<td>P K</td>
<td>05.99</td>
<td>09.32</td>
</tr>
<tr>
<td>CIK</td>
<td>10.47</td>
<td>14.24</td>
</tr>
<tr>
<td>AgL</td>
<td>61.03</td>
<td>27.27</td>
</tr>
<tr>
<td>CaK</td>
<td>10.29</td>
<td>12.37</td>
</tr>
</tbody>
</table>

**Table 5**

**Figure 18a.**
Regarding subgroup 3:

Figure 18a: Backscattered electron image of SEM (a) and corresponding EDX spectrum (b) of the fractured surface of resin dentin beam side at a magnification 1000x. Figure 18b: Element profile for Filtek™ Z 250 + G-Bond plus (Etch and rinse mode) with hydrophobic resin coating.

Table 6: Amount of silver at energy level (L) in Figure 19a. SEM and EDX analysis

Figure 19a: is a backscattered electron image of the morphology and surface composition of the resin-dentin beam side of the fractured surface of a specimen. The granules of silver depositions (b) in the hybrid layer of the resin dentin beam show that silver uptake was (9.28 wt %) in Table 6 at energy level (L).

Regarding subgroup 4:

Figure 20a: Backscattered electron image of SEM (a) and corresponding EDX spectrum (b) of the fractured surface of resin dentin beam side at a magnification 1000x. Figure 20b: Element profile for Filtek™ Z 250 + G-Bond plus (Etch and rinse mode) without hydrophobic resin coating.

Table 7: Amount of silver at energy level (L) in Figure 20a. SEM and EDX analysis

Figure 20a: is a backscattered electron image of the morphology and surface composition of the resin-dentin beam side of the fractured surface of a specimen. The granules of silver depositions (b) in the hybrid layer of the resin dentin beam show that silver uptake was (11.84 wt %) in Table 7 at energy level (L).

4. Discussion

4.1. For Microtensile

Regarding the effect of G-enial bond plus (GBP), is a HEMA-free 1-step 4-MET-derived self-etch adhesive, with a pH 1.5 [24]. GBP is not recommended in ER mode on dentin. However, we decided to experimentally apply
GBP on phosphoric acid etched dentin, as some etching gels may inadvertently overflow to dentin during clinical procedures when selective enamel etching is used. GBP/SE resulted in statistically greater mean TBS compared to GBP/ER without HC, which validates the respective manufacturer’s recommendation for not etching dentin, as well as, recently showed in the literature [24]. Regardless of the application mode, GBP had the poorest performance even when coated with HC. These findings are in agreement with previous studies [25]. Although Hanabusa et al. reported similar bond strengths for ER and SE strategies; they also reported low-quality hybridization in the ER mode for GBP, specifically a resin-infiltrated collagen network with signs of adhesive incomplete infiltration [25]. The paradox is that, to reach the acidic pH that allows the self-etching capability, hydrophilic properties cannot be avoided. In fact, acidic self-etch formulations (low pH as in GBP) need more hydrophilic and acidic resins blends [26]. The relatively low pH of 1.5 in GBP allows a more aggressive enamel and dentin demineralization [27], with less hydroxyapatite available for chemical interaction with the 4-MET, resulting in lower mean µTBS. If compared with the 10-MDP functional monomer, 4-MET is less hydrolytically stable [28], which also applies to the resulting 4-MET calcium complexes [29]. The 4-MET functional monomer is not able to chemically interact with calcium in hydroxyapatite through nanolayering [29]. The lack chemical bonding to calcium by 4-MET may have been responsible for the lower mean µTBS of GBP/SE compared to those of GBP/ER. Furthermore, GBP has acetone as organic solvent, which might contribute to a higher susceptibility to the degree of moisture in dentin [26].

SBU is HEMA-containing adhesives (Figure 2), in opposition to GBP, which is a HEMA-free adhesive. HEMA is a hydrophilic monomer added to self-etch adhesives to enhance dentin wettability and monomer infiltration [30] and prevent hydrophobic monomer/water phase separation [31]. The incorporation of poly-HEMA in the polymer net-work enhances water uptake after polymerization [30], due to poly-HEMA hydrolytic degradation and elution of by-products during long-term storage [32]. HEMA-containing adhesives are more hydrophilic and have higher water sorption [33]. In long-term water storage, the reduction in the tensile strength of adhesives increases with their hydrophilicity, reducing their mechanical properties [34]. The exclusion of HEMA within GBP composition has been suggested to have the potential of reducing the adhesive hydrophilic properties and, consequently, to avoid the decline in mechanical properties due to water sorption [32]. Water sorption and ultimate tensile strength of HEMA-free adhesives do not significantly change with water storage [32]. However, HEMA-free formulations do not produce bond strengths as higher as those of HEMA-containing adhesives [35], which is in agreement with the results of our study.

4.2. For Nanoleakage

Regarding hydrophobic resin coating vs. non coating, in our study, HC resulted in greater mean µTBS of SBU/ER and GBP/SE. The thickness of the adhesive layer may have increased [36] allowing the formation of a more densely packed hybrid layer with improved mechanical properties and nanoleakage characteristics. The HC also increased the mean µTBS of SBU/ER and GBP/SE, which may have been a result of enhanced adhesive layer hydrophobicity. The adhesive layer becomes less permeable to water movement, and less susceptible to water degradation [36]. Coating with a hydrophobic layer may couple more un solvated hydrophobic monomers to the adhesive interface through copolymerization with the uncured adhesive surface, decreasing the relative concentration of retained solvent and unreacted monomers, thus enhancing the in situ degree of conversion. In our study, regarding SBU/ER and GBP/SE, exhibited the least amount of silver granules deposits at the bottom of the hybrid layer (6.9-7.6%). The performance of GBP improved after the application of HC. The GBP’s inherent hydrophilic nature may have been reduced by HC allowing a higher in situ degree of conversion [37]. However, we observed severe increase of NL within the hybrid layer for GBP/SE, which may have been a result of the hydrolysis of the phosphoric acid ester monomer, which may have caused dentin demineralization over time. The instructions for use of GBP may have to be revised. The instructions for use SBU clearly stated that this universal adhesive must be applied actively, with two consecutive coats. Active application [37,38], double application [39], and a greater infiltration time [40] are known to improve the performance of self-etch adhesives. The manufacturer of GBP recommends applying GBP passively, with 10 s of infiltration compared to 20 s for SBU, which may have adversely affected the interaction of GBP with dentin. GBP also resulted in higher nanoleakage with a water-tree pattern in the adhesive layer, characteristic of HEMA-free adhesives due to phase separation and residual water on the dentin surface [41]. The dendritic pattern may also have been a result of phase separation.

When we analyzed the NL results for ER and SE, we were unable to find a cause–effect relationship from the application of HC, as it occurred for µTBS. Some reductions were observed within groups with HC (SBU/ER, GBP/SE). NL may be more related to the adhesive infiltration and sealing capability. It is well known that the quality of the resin–dentin bonds is affected by the extent of resin infiltration into the exposed collagen [40,41]. For ER, peritubular hybridization of the resin tags may not occur. For SE, the weakest zone in aged specimens is below the hybrid layer, due to poorer polymerization of the monomers within the bottom of the hybrid layer [42]. These findings corroborate our NL pattern observations for both ER and SE modes. Even if resin hydrolysis may negatively affect the long-term bonding stability, collagen depletion may also occur due to enzymatic degradation. The activation of matrix metallo-proteinases (MMP’s) is induced by adhesive chemical formulations on both mineralized and demineralized dentin, regardless of the bonding strategy [43]. However, MMP’s degradation is believed to be more destructive for ER hybrid layers than for mild SE hybrid layers, as SE adhesives bond to dentin with less demineralization [44].
5. Conclusion

An extra hydrophobic layer coating improved the immediate in vitro performance (µTBS and NL) of the universal adhesive systems that studied in SE mode. However, NL pattern is material-dependent and aging stability seems not to be related with the adhesive strategy.

References

