Microscopy comparative evaluation of the SE systems adhesion to normal and sclerotic dentin

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Abstract
The purpose of this study was in vitro evaluation and comparison of the adhesion of self-etch (SE) adhesive systems applied on normal and sclerotic dentin. For this study, Class 5 cavities were prepared on sound teeth as well as on teeth with sclerotic dentin. They were then restored by means of the SE 2-step OptiBond XTR (Kerr) and SE 1-step Bond Force (Tokuyama Dental) adhesive systems, as well as the Estelite Sigma Quick (Tokuyama Dental) composite resin. For teeth with sclerotic dentin, the hypermineralized superficial layer was removed by means of round bur on low speed, than the adhesive systems and composite resin were applied. These teeth were prepared for microscopic study according to the protocol specific to each microscope. SEM (scanning electron microscopy) examination reveals that on normal and sclerotic dentin, OptiBond XTR and Bond Force form hybrid layers with about the same thickness, greater in normal dentin, but only OptiBond XTR pervades into the dentinal tubules, both in normal and sclerotic dentin. However, TEM (transmission electron microscopy) examination of Bond Force reveals that it penetrates into the dentinal tubules as well, but only in the case of normal dentin. The thickness of the hybrid layers resulting from the application of the SE adhesive systems to sound dentin is different from the thickness of the hybrid layers obtained when the same adhesive systems have been applied to sclerotic dentin.

Keywords: sclerotic dentin, SE 2-step, SE 1-step adhesive systems, hybrid layer, hybridized complex.

\(\Delta\) Introduction
Sclerotic dentin, formed in the areas where it is unprotected shows essential morphological alterations, which consist in partial or total obliteration of the dentinal tubules through mineral deposits in addition to the peritubular apposition. Dentin becomes transparent as well as insensitive, while the lesions surface appears smooth [1]. The thickness of the sclerotic dentin layer is variable.

Sclerotic dentin presents alterations of its composition. Studies have shown the presence of a small percentage of Mg (4.57%) in the composition of sclerotic casts, thus confirming their being composed of whittlockite [2].

Another morphological alteration of the sclerotic dentin decelerated through TEM (transmission electron microscopy) occurs on the surface of the sclerotic dentin of the wedge-shaped lesions of a hypermineralized layer; this layer withstands theetching action, in turn preventing the formation of the hybrid layer necessary for the adhesion process [3]. The hypermineralized layer has an uneven surface and variable thickness. On the occlusal wall, the hypermineralized layer is 1–2 μm thick, while, on the gingival wall, it is approximately 200–300 nm [4].

In the deepest part of the lesion, the hypermineralized layer is 15 μm thick, inaccessible for brushing, and contains bacteria. According to Tay & Pashley, the hypermineralized layer contains two types of bacteria, one on its surface and one in between its layers. This indicates that the changes in the oral cavity micro-flora may lead to periodic bacterial colonization. Each bacteria colony has been mineralized before the next colony is deposited. The metabolism of microorganisms produces substantial pH fluctuations along the dental surface. Bacterial metabolic products may trigger a gingival inflammation together with an increase in the flow of gingival liquid, which in turn nurture the microorganisms [3].

These morphological alterations result in a decreased efficiency of the adhesives applied to sclerotic dentin. The efficiency of total-etching adhesive systems has been proven; they have a “golden standard” status. However, the use of self-etch (SE) adhesive systems shows some advantages that are as follows: decrease of working time; maneuverability; mistakes in the laying technique are avoided through removal of supplementary working stages [5] and aspects in relation to the efficiency of these simplified adhesive systems (SE) still need to be clarified, as very few published data are available on their clinical efficiency on sclerotic dentin lesions [6]. Also, adhesion to the unprepared sclerotic dentin is less predictable.

The aim of the study is to highlight the hybrid layer resulting from the two adhesive systems and to compare the aspect and thickness of the layers formed on sound and sclerotic dentin.

\(\Delta\) Materials and Methods
For this study, seven sound teeth, as well as seven teeth with sclerotic dentin, which had been extracted for periodontal reasons, were used. These teeth had plaque and soft tissue removed, were brushed with prophylactic paste.
(Clean Polish, Kerr), kept in saline solution for 24 hours in a refrigerator, and then in chloramine T/formalin.

Preparation protocol for sound teeth

Class 5 cavities were prepared and then restored by means of the SE 2-step OptiBond XTR (Kerr) and SE 1-step Bond Force (Tokuyama Dental) adhesive systems, which were applied according to the protocol recommended by each producer.

For OptiBond XTR, the primer is applied to the enamel and the dentin by a brushing movement for 20 seconds, then dried for 5 seconds. The bonding is applied to the enamel and the dentin by gentle brushing for 15 seconds, then gently dried for 5 seconds and finally light-cured for 10 seconds.

For Bond Force, the adhesive system is applied to the surface of the tooth by brushing for 20 seconds, gently dried for 5 seconds and then strongly dried for 5 seconds; finally, light-curing for 10 seconds.

Estelite Sigma Quick (Tokuyama Dental), which is a nanocomposite with reduced shrinkage recommended in cavities I–V restorations, was used. This composite offers an improved working time of 90 seconds under ambient light, as well as a curing time reduced by 10 seconds when a halogen lamp is used.

Preparation protocol for teeth with sclerotic dentin

The same adhesive systems, as well as the same composite resin were used with teeth with sclerotic dentin, once the hypermineralized superficial layer had been removed from its surface, according to the same protocols recommended by each producer.

Preparation protocol for SEM examination

The sections intended for study with the Scanning Electron Microscope (SEM) were embedded in self-curing acrylic resin.

Preparation protocol for TEM examination

The 2 mm³ decalcified pieces used in electron microscopy were set in 5% glutaraldehyde, then set with 1% osmium tetroxide, contrasted in 1% uranyl acetate, and dehydrated in series of ethanol. Then, they were infiltrated in propylene-oxide, wrapped in Epon 812 resin and sectioned with the ultramicrotome (Leica UCT, Austria). Ultra-sections (100 nm in width) were mounted on copper grids (300 mesh), dyed with lead citrate and 2% uranyl acetate, observed and photographed by means of a LEO 912 transmission electron microscope (Oberkochen, Germany).

Results

SEM examination

SEM examination of the adhesion to sound dentin of OptiBond XTR adhesive system reveals the formation of a hybrid layer, which, at the DEJ (dentin-enamel junction) level, is of a varying thickness measured on probes, between 20–60 μm, while at the level of the dentin between 10–30 μm. Also, it is observed to penetrate the dentinal tubules (Figures 1 and 2).

The same examination of Bond Force adhesive system shows the formation of a hybrid layer, which, at the DEJ level, is of a varying thickness, between 6–16 μm, while at the dentin level of 4–30 μm. Also, it is observed not to penetrate the dentinal tubules (Figures 3 and 4).

SEM examination of OptiBond XTR adhesive applied to the sclerotic dentin shows a hybrid layer of 2–4 μm thickness measured on many probes, while, most importantly, it is observed that the adhesive penetrates the dentinal tubules for a distance of 4 μm (Figures 5 and 6).

Following the compositional and quantitative analysis corresponding to the EDS spectrum of the interface tooth–restoration material, the presence of Mg, an element characteristic of the sclerotic dentin, is observed (Figure 7; Table 1).

During SEM examination of Bond Force adhesive system–sclerotic dentin interface, it could be noted that a 3 μm hybrid layer is formed, but the adhesive does not penetrate the dentinal tubules (Figures 8 and 9).
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Figure 3 – The interface restoration material–dentin, 500× magnification.

Figure 4 – Detail of the interface adhesive–dentin, 2000× magnification.

Figure 5 – The interface restoration material–dentin, 500× magnification.

Figure 6 – Detail of the interface adhesive–dentin, 2000× magnification.

Figure 7 – X-radiation emission spectrum corresponding to the analysis of the tooth–restoration material interface layer.
Table 1 – Results of the compositional and quantitative analysis corresponding to the EDS spectrum

<table>
<thead>
<tr>
<th>Element</th>
<th>Wt %</th>
<th>At %</th>
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<tbody>
<tr>
<td>C</td>
<td>60.82</td>
<td>72.95</td>
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<tr>
<td>O</td>
<td>21.58</td>
<td>19.43</td>
</tr>
<tr>
<td>Na</td>
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<td>0.31</td>
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<tr>
<td>Mg</td>
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</tr>
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<td>Total</td>
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</tr>
</tbody>
</table>

EDS: Energy Dispersive Spectroscopy; Wt: Weight; At: Atom.

TEM examination

TEM examination of the adhesion to sound and sclerotic dentin of OptiBond XTR adhesive system did not occur because of the detachment of the adhesive layers from the dental probes during the quick decalcification procedure.

Therefore, hybrid layers resulting from the application of the OptiBond XTR adhesive system are highlighted and measured only by means of SEM.

The TEM examination in a bright field, at 80 kV, of the adhesive–normal dentin interface confirms the formation of a hybrid layer and reveals the penetration of the adhesive into the dentinal tubules, when Bond Force adhesive system is used. This examination has been carried at progressive enlargements of 6300×, 16 000× and 31 500× (Figures 10–12).

TEM examination in a bright field, at 80 kV, of the adhesive (Bond Force)–sclerotic dentin interface highlights the presence of the hybridized complex, consisting in the adhesive, the hybridized smear layer and the less-present hybrid layer. The adhesive does not penetrate the dentinal tubules. The examination has been carried out at enlargement of 6300× and 10 000× (Figures 13 and 14).

Figure 8 – The interface restoration material–dentin, 500× magnification.

Figure 9 – Detail of the interface adhesive–dentin, 2000× magnification.

Figure 10 – TEM appearance of the hybrid layer formed by the Bond Force, 6300× magnification. The adhesive is observed to penetrate the dentinal tubule.

Figure 11 – TEM appearance of the hybrid layer formed by the Bond Force, and penetration of the dentinal tubule, 16 000× magnification.

Figure 12 – TEM appearance of a dentinal tubule, that has been penetrated by the Bond Force adhesive, 31 500× magnification.
Discussion

Generally, studies on the efficiency of dental adhesives compare the SE systems to the ER (etch-and-rinse) systems, which have been considered to be the best up to the present time. SE adhesives systems are not based on a separate etching step like ER systems; the adhesive contains an acid monomer, which is not removed through rinsing, the smear layer becomes permeable, and thus it is incorporated into the hybrid layer.

The studies have mainly been applied to sound enamel and dentin. It was the tendency to simplify the adhesive techniques, represented by the advent of SE adhesives, as well as the relatively scarce information on the behavior of SE systems applied to prepared/unprepared sclerotic dentin that have led to the present research.

Sclerotic dentin without instrumentation can influence adhesion due to the specific pathological conditions, such as: presence of sclerotic deposits in dentinal tubules, which block formation of resin tags, existence of the hyper-mineralized layer and of bacteria [7].

That is why, in this study, a superficial layer was removed by means of a low speed round bur.

During the SEM examination of several sclerotic dentin samples on which the Bond Force adhesive system was applied, it was observed that the adhesive does not penetrate into the dentinal tubules, which stay obliterated with sclerotic casts and the smear layer; the hybrid layer thickness is approximately 3 μm. It is to be noted that before applying the adhesive system, there was no 37% phosphoric acid etching. Lack of the adhesive in the dentinal tubules indicates the need for predemineralization of the sclerotic dentin with phosphoric acid so as to increase the clinical efficiency of the adhesive system in this area.

Bond Force adhesion to normal dentin is much better than to sclerotic dentin, and was evaluated with the SEM and TEM without the pre-etching of the dentin. In the SEM image, the formation of a thin hybrid layer of approximately 2 μm (the whitish area) can be observed, with the adhesive layer thickness of 20–40 μm. On the TEM image, the existence of a well-represented hybrid layer on the dentin surface can be noted; the adhesive penetrates into the dentinal tubules.

OptiBond XTR has been examined only with the SEM and it has been observed that when it is applied to normal dentin it forms a well-represented hybrid layer and penetrated the dentinal tubules.

When samples of interface of sclerotic dentin–OptiBond XTR have been examined, the hybridized layer is noted to be much thinner than the one formed on normal dentin, but, most importantly, the adhesive is observed to penetrate the dentinal tubules. Thus, if the two adhesive systems are compared, it may be said that adhesion to normal and sclerotic dentin of the SE 2-step OptiBond XTR adhesive system is stronger than in the case of the SE 1-step Bond Force adhesive system.

There are differences between the thickness of the hybrid layers obtained by means of the SE systems applied to normal and sclerotic dentin, which supports the observation that adhesion to sclerotic dentin is less strong than to normal dentin.

The same results were observed when another SE 1-step adhesive system (Futurabond M, VOCO) was applied to sound or sclerotic dentin. The study concluded that the adhesion of SE 1-step systems to sclerotic dentin features particular aspects as the hybrid layer is very thin compared to the normal dentin, while the adhesive does not pervade into the dentinal tubules [8]. Thus, the dentin type influences the quality of the adhesion. This fact is maintained by Bulucu et al. [9] who compare micro-tensile bond strengths of three different adhesive systems applied on sound human and bovine dentin, caries-affected dentin and dentin of unerupted human teeth.

Micro-tensile bond strengths evaluates the quality of the adhesion and is the method who has been developed by Sano et al. and is called the mTBS test. This technique allows the use of smaller, uniform dentin samples. With this method, less number of teeth is required [10].

The authors have compared the ER, SE 2-step and SE 1-step adhesive systems and observed that statistically similar bond strength was obtained with ER adhesive system and SE 2-step, while SE 1-step showed the lowest bond strengths. Mean bond strength to sound human and bovine dentin was significantly higher than to caries-affected dentin and dentin of unerupted human teeth [9].

The reduced efficiency of an SE 1-step adhesive system
has been demonstrated by Nishimura et al., who have measured the micro-tensile bond strengths one year after application of the adhesive to the teeth with wedge-shaped defects. The conclusion of the study was that the tensile bond strengths of the all-in-one adhesive decreased over one year [11].

Studying the bond strengths of different adhesive systems, Karakaya et al. have also found that with sound dentin, SE 2-step adhesive system showed a significantly higher bond strength than the other adhesives ($p=0.05$). With sclerotic dentin, although there were no significant differences in bond strength among the studied adhesives, the bond strength values of SE 2-step and ER adhesive systems were significantly decreased [12].

It appears that the therapeutic attitude regarding sclerotic dentin needs to include the removal of a superficial layer on its surface by means of a low speed round bur, as well as pre-etching with 37% phosphoric acid in the case of employing simplified SE 2-step or SE 1-step adhesive systems in order to obtain a better adhesion to this substrate [7, 13].

A study by Eliguzeloglu et al. [13] has shown that the hybrid layer thickness increases when a superficial layer is removed off the surface of sclerotic dentin. Association of the SE 1-step technique with the removal of a layer off the surface of the sclerotic dentin and with demineralization with 37% phosphoric acid results in an increase of the hybrid layer thickness [13, 14].

The recommendation to remove a superficial layer off the sclerotic dentin has been made by Hedge [4] as well, who says that although sclerotic dentin is part of the natural defense system of the organism and should be kept whenever possible, the best way to restore a lesion is through removing a thin layer on the surface of the hypermineralized dentin, this way removing the adherent bacteria layer on the surface, too.

However, Luque-Martinez et al. do not recommend removing it with a diamond burs, irrespective of their roughness [15]. The use of diamond burs with different roughness did not increase the bond strength of self-etch systems and etching pattern in sclerotic dentin. Clinicians should avoid using this procedure when applying self-etch adhesive [15].

### Conclusions

During SEM examination, the thickness of the hybrid layers resulting from the application of the SE 2-step OptiBond XTR (Kerr) and SE 1-step Bond Force (Tokuyama Dental) adhesive systems to normal dentin is different from the thickness of the hybrid layers obtained when the same adhesive systems have been applied to sclerotic dentin. The thickness of the hybrid layers obtained with SE adhesive systems have higher values in the case of sound dentin, but the Bond Force adhesive system does not seem to penetrate into the dentinal tubules, a fact that is contradicted by the TEM examination, which reveals that this system penetrates into the dentinal tubules as well, but only in the case of normal dentin. The values of hybrid layer thickness are lower for sclerotic dentin, a fact that supports the clinical observations according to which adhesion to sclerotic dentin is weaker than to normal dentin, ultimately influencing the longevity of dental restorations.

### Conflict of interests

The authors declare that they have no conflict of interests.

### References


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