Microscopic aspects of the hybrid layer formed by the SE 1-step Futurabond M (Voco) adhesive system applied to normal and sclerotic dentin

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Abstract

Study objectives: In vitro evaluation and comparison of the adhesion of a generation-7 adhesive system to normal and sclerotic dentin. Materials and Methods: For this study, sound teeth as well as teeth with sclerotic dentin, which had been extracted for periodontal reasons, were prepared. Class 5 cavities were prepared, then restored by means of the SE 1-step Futurabond M (Voco) adhesive system, 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, then the adhesive system and composite resin were applied. These teeth were prepared for microscopic study according to the protocol specific to each microscope. For the study involving the confocal microscope, the adhesive was mixed with the Evans Blue dye before being applied to the tooth, then the same protocol was followed. Results: When applied to normal dentin, Futurabond M (Voco), the generation-7 adhesive system, forms a hybrid layer with a depth of 20–25 μm, while it can be noted that it pervades 6–8 μm into the dentinal tubules. When applied to sclerotic dentin, it was noted that the adhesive system does not pervade into the tubules, with an approximately 10–15 μm depth of the hybrid layer. Conclusions: The adhesion to sclerotic dentin shows particular aspects. When it is desired to employ generation-7 adhesive systems (SE 1-step) on sclerotic dentin, the therapeutic approach needs to include the following supplementary stages: removal of the superficial hypermineralized layer, as well as predemineralization with 37% phosphoric acid; they are the only stages that might improve the adhesion to this substrate.

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

Introduction

Patients’ requests for aesthetic restorations are at present supported by the developments in the field of dental materials, usage of composite resins presenting an aesthetic potential, by the possibility of a minimally invasive treatment (by comparison to the silver alloy restorations), by the maneuverability and ability to withstand mechanic wear. Nevertheless, clinical success of composite resin restorations depends on the morphological substrate (enamel, normal dentin/sclerotic dentin/cement), as well as on the adhesive system type or the shape of the cavity.

Up to present day, adhesion to dentin remains a challenge by comparison to the adhesion to enamel, owing to the complex, “hybridized” structure in the case of normal dentin, or to the highly mineralized structure in the case of sclerotic dentin. Adhesion to enamel is ensured by a uniform adhesive substrate, because of the pervasion of the adhesive resin to microretentions resulting from the demineralization process. Dentin is a complex substrate owing to its tubules-like structure and composition [less than 50% inorganic material and high content of water (21%)]. Adhesion to dentin also depends on its type (normal or sclerotic) [1, 2].

The sclerotic dentin substrate presents difficulties when using adhesives owing, on the one hand, to the sclerotic casts in the dentinal tubules, and, on the other hand, to the hypermineralized layer on its surface, a layer which presents bacterial inclusions, damaged collagen and large mineral crystals [3, 4]. Nevertheless, SE-adhesive systems do have their own limitations. Knowing these limits ensures the correct choice of the most appropriate system depending on the clinical case, and results in a predictable treatment.

This study aims to present the microscopic aspect of the hybrid layer resulting from the use of an SE 1-step adhesive system on teeth with normal and sclerotic dentin.

Materials and Methods

For this study, 10 sound teeth, as well as 10 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, which were then restored by means of the SE 1-step Futurabond M (Voco) adhesive system, which was applied according to the protocol recommended by the producer (apply the adhesive...
to the dental surface, wait for 20 seconds, gently dry for five seconds, and light-cure for 10 seconds) and Estelite Sigma Quick (Tokuyama Dental), a nanocomposite with reduced shrinkage recommended in cavities I–V restorations. This composite is based on the RAP (Radical-Amplified Photopolymerization) initiator technology and 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 system, 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.

The composition of the materials used is found in Tables 1 and 2.

**Table 1 – Composition of the adhesive system**

<table>
<thead>
<tr>
<th>Adhesive</th>
<th>Laying technique</th>
<th>Filling</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Futurabond M (Voco)</td>
<td>all-in-one</td>
<td>Microfilled-SiO2 particles of 20 nm diameter</td>
<td>Urethane dimethacrylate 20–25%</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td></td>
<td>• Ethanol 10–25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Monomer acidic adhesive 5–10%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 2-Hydroxyethyl methacrylate 2.5–5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Catalyst, less than 2.5%</td>
</tr>
</tbody>
</table>

For examination with the confocal microscope, a mixture of adhesive and Evans Blue dye (Sigma E2129, Germany) was applied to several samples, followed by light-curing, and the restoration was carried on. After finishing and polishing, the teeth were sectioned longitudinally by means of fine diamond discs active on both sides, and samples for microscopic study were prepared.

**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 light, fluorescent and confocal microscopy**

Other 5 mm-thick sections removed from the areas that presented interest for the study were set in 10% formaldehyde for three days, and then kept in a quick-decalcifying solution (Biodec R, Italy) for a week. The decalcified samples, intended for fluorescent and confocal optical microscopy study, were immediately frozen by submerging them into liquid nitrogen. Twenty μm cryosections were made in a coronal plane by means of a cryotome (Leica CM1850, Germany), then mounted on slides. Sections were dyed by means of the classic method with Hematoxylin–Eosin (HE), as well as through the Schmorl’s method with picrothionin for hard tissues. The slides were evaluated by means of a Carl Zeiss Axioplan 2 microscope equipped with a Canon Power Shot G3 digital camera and a Carl Zeiss LSM 510 laser scan module (Carl Zeiss GmbH, Oberkochen, Germany).

**Preparation protocol for TEM examination**

The 2-mm³ decalcified pieces used in electronic 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 (Carl Zeiss, Oberkochen, Germany).

**Results**

**Light and fluorescence microscopy evaluation examination**

Dentin normally highlighted by means of the HE staining presents a homogenous dentinal tubules structure. Demarcation between the dentin and the adhesive is partially interrupted, probably because of the sampling technique. The establishment of a hybrid layer, covered by the adhesive layer, is observed on the surface of normal dentin (Figure 1).

The picrothionin dye highlights the dentin in yellow and the tubules in light brown. The adhesive layer is brown dyed and the dentin hybridization can be observed (Figures 2 and 3).

The Evans Blue (EvB) dye was used to better delineate the hybrid layer and adhesive one (Figures 4 and 5). Epifluorescence examination has been achieved using HBO lamp mounted on microscope and 560/620 nm excitation/emission filter. Establishment of the hybrid layer/hybridized complex is highlighted with all samples.

**Confocal microscopy examination**

Confocal microscopy examination was done by means of a single laser source (HeNe). Establishment of a hybrid layer with a uniform width of 20–25 μm can be observed during confocal microscopy examination (Figure 6).

**SEM examination**

**Sound dentin evaluation**

During the SEM examination of the Futurabond M (Voco) adhesive system on normal dentin, it was observed that dentin was hybridized, while the adhesive system pervades 6–8 μm into dentinal tubules (Figures 7 and 8).

**Sclerotic dentin evaluation**

When the Futurabond M (Voco) adhesive system is applied to sclerotic dentin, it was observed that it does not pervade into dentinal tubules; the tubules stay...
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obstructed by the smear layer sclerotic deposits. The area containing hybridized dentin seems to be very thin (Figures 9 and 10).

**TEM examination**

**Sound dentin evaluation**

TEM examination in a bright field, at 80 kV, of the adhesive-normal dentin interface confirms the formation of a hybrid layer and pervasion of the adhesive into the dentinal tubules. Microfilling particles from the adhesive system composition are also observed; they pervade into the dentinal tubules, thus suggesting the establishment of a resistant hybrid layer (Figures 11–14).

**Futurabond M aspect – sclerotic dentin**

TEM examination in a bright field, at 80 kV, of the adhesive-sclerotic dentin interface highlights the hybridized complex made up of the adhesive, hybridized smear layer and a very thin hybrid layer. The adhesive does not pervade into the dentinal tubules. Examination was done at progressive magnification ratios of ×6300 and ×8000 (Figures 15 and 16).

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**Figure 1** – Optical microscopy aspect of the normal dentin region (HE staining): hybrid layer-adhesive dyed with Evans Blue (EvB), ×10.

**Figure 2** – The same dentin-hybrid layer-adhesive aspect in the case of a picrothionin-dyed specimen, ×10.

**Figure 3** – Adhesive–dentin interface aspect, HE staining (×20), in field of view. Red – dentin; black – hybrid layer; blue – Futurabond M mixed with EvB.

**Figure 4** – The same aspect, seen in epifluorescence. (A) Concentration of fluorescent dye (EvB) is seen in the adhesive. Dentin has a basal self-fluorescence level, ×20.

**Figure 5** – Histogram representing the EvB dye fluorescent signal intensity of the hybrid layer and the adhesive.

**Figure 6** – Confocal microscopy image: adhesive/hybrid layer/normal dentin; adhesive width 35–40 μm, hybrid layer width 20–25 μm.
Figure 7 – Restoration material–dentin interface when enlarged ($\times$500).

Figure 8 – Detail of the adhesive–dentin interface when enlarged ($\times$2000); adhesive layer width 10–25 $\mu$m.

Figure 9 – Restoration material–dentin interface when enlarged ($\times$500): other aspects.

Figure 10 – Detail of the adhesive–dentin interface when enlarged ($\times$2000): other aspects.

Figure 11 – TEM aspect of the hybrid layer formed by the Futurabond M adhesive ($\times$5000).

Figure 12 – TEM aspect of the hybrid layer formed by the Futurabond M ($\times$6300): pervasion of the filling particles into the dentinal tubules is observed.
Most studies evaluate the adhesion of SE systems from a clinical point of view, by following criteria such as marginal adaptation (restorations employing SE 1-step adhesive systems present a good marginal adaptation six months from their application [5], but also marginal degradation at the enamel on the 12-month evaluation [6]; This could highlight the fact that SE adhesive systems are not able to form microretentions well represented on the enamel because of the weaker monomer acids in their composition), marginal discoloration (it was observed that 18 months later marginal coloring on the enamel is poor in the case of SE adhesive systems [7, 8], therefore marginal adhesion is poor; the same marginal coloring has also been observed when evaluating certain restorations 18 and 36 months later, and again it was the case for the SE adhesive systems [8, 9]), retention (clinical studies report a good retention rate of restorations both in the case the selective etching of the enamel is not performed [10], and when it is [11]).

The data provided by these clinical studies are limited, and the results are based on variables such as following the protocols for the adhesive systems application or on interpreting the date in a different manner by various examiners. The shortcomings of SE adhesive systems may thus be sensed/deducted, but they are not supported by clear evidence. In scientific literature, there are a few microscopy studies which to present the hybrid layer/the hybridized complex established by the SE systems when they are applied to sclerotic dentin.

SE 1-step adhesive systems can be used in restoring various cavities, especially in areas less subjected to occlusive stress, as the hybrid layer formed when they are applied to normal dentin is a predictable one. The same cannot be said when the same systems are applied on sclerotic dentin, where, as a result of the lack of instrumentation, the adhesive meets a hypermineralized layer of a variable width which it cannot pervade into, while in the case of instrumentation of sclerotic dentin, the obstacle is the altered smear layer resulting from whitlockite crystals and hypermineralized dentin, both resistant to acid [12].

In this study, following analysis of the normal dentin–adhesive and the sclerotic dentin–adhesive interfaces, the establishment of the hybrid layers can be observed in both situations, as well as that of resins tags approxi-
mately 6–8 μm in length when the adhesive system is applied to normal dentin. This is also supported by Eliguzeloglu et al. [13] who have also observed that SE 1-step adhesive systems may form resin tags with sideways thin and sparse ramifications when they are applied to sclerotic dentin where the superficial layer had been removed, and that there are no significant differences between the width of hybrid layers established by SE 1- and 2-step adhesive systems when superficial dentin is not removed [13].

By in vitro evaluation of different aspects of the adhesion to sclerotic dentin obtained by means of SE adhesive systems, the majority of the authors support the removal of the hypermineralized superficial layer and the pre-etching of sclerotic dentin with 37% phosphoric acid [4, 14, 15].

Conclusions
The adhesion of SE 1-step systems to sclerotic dentin shows particular aspects as the hybrid layer is very thin comparing to the normal dentin and the adhesive does not pervade into the dentinal tubules.

References

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