

Article

SEM Evaluation of the Hybrid Layer of Two Universal Adhesives on Sound and DI Type II Affected Dentin

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Abstract: Universal nanofilled adhesives were recently introduced in restorative dentistry to simplify clinical procedures and improve adhesion in different clinical situation. This study investigated the effectiveness of two universal adhesives on both sound and dentinogenesis imperfecta type II (DI-II)-affected teeth. To evaluate the effectiveness of adhesion on both sound and DI-II-affected teeth, four samples, two sound extracted molars and two extracted molars affected by DI-II were selected. Coronal enamel and dentin were exposed, and the samples were used for testing two different universal adhesives, Universal Bond (Tokuyama) and All-Bond Universal (Bisco). After the adhesive procedures, the samples were stored in saline at room temperature for one week prior to SEM investigation for the interfacial bonding layer. The samples were longitudinally sectioned into two parts, obtaining two sections for the evaluation of the adhesive interface to the SEM. The SEM-morphology of the hybrid layer on the enamel was similar for the two universal adhesives tested. The study of the hybrid layer on sound dentin confirmed the great versatility of All-Bond Universal and Universal Bond adhesives in managing adhesion even on pathological dental substrates. Both universal adhesives tested showed encouraging results on DI-II-affected dentin, creating an effective hybrid layer even on the atubular and less mineralized altered dentin.

Keywords: universal adhesive; dentinogenesis imperfecta type II; SEM analysis; dentin bonding; nano-hybrid composite resin



Citation: Libonati, A.; Di Taranto, V.; Memè, L.; Gallusi, G.; Campanella, V. SEM Evaluation of the Hybrid Layer of Two Universal Adhesives on Sound and DI Type II Affected Dentin. *Appl. Sci.* **2022**, *12*, 11372. <https://doi.org/10.3390/app122211372>

Academic Editor: Andrea Scribante

Received: 3 October 2022

Accepted: 7 November 2022

Published: 9 November 2022

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1. Introduction

In the last 20 years, universal adhesive systems have been introduced into dentistry. This type of bonding system, also known as “multi-modal”, since they can be used in etch-and-rinse, self-etch (single-step), selective enamel etching and on virtually any kind of dental or prosthetic substrate, simplifies operative procedures significantly by reducing steps and the need for different adhesives for different substrates [1–3]. Exclusively in relation to the intended use, these kinds of adhesives are similar to older and now-surpassed single-step adhesives [4].

The universal adhesives contain hydrophilic and hydrophobic monomers, polymerization promoters, solvents, stabilizers and fillers.

The composition is similar to the self-etch all-in-one adhesives, except for the presence of carboxylate or phosphate monomers, as 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate) are capable of forming ionic bonds with the calcium of the hydroxyapatite and forming insoluble calcium salts [5]. The 10-MDP is a hydrophilic monomer with mild-etching properties and allows universal adhesive to be used with any type of etching technique [4,6]. Universal adhesives also contain biphenyl dimethacrylate (BPDM), dipentaerythritol pentaacrylate phosphate acid ester (PENTA) and polyalkenoic acids, which improve adhesion in terms of durability, reducing water absorption and the hydrolytic

degradation of the adhesive interface. Hydrolytic degradation represents one of the main causes for delayed adhesive bonding failure [7,8].

Universal adhesives also contain hydrophilic monomers (HEMA-hydroxyethyl methacrylate), hydrophobic (D3MA—decenediol dimethacrylate) and intermediates, such as bis-GMA (bisphenol A-glycidyl methacrylate) meant for the creation of bonding bridges between the hydrophilic surface of the tooth and the hydrophobic surface of the resin. Finally, most universal adhesives contain silane, whose function is to facilitate adhesion to glass-ceramic substrates. The universality of these products made them a viable option in many different applications for direct and indirect restorations. Despite the improvements shown in simplified techniques, universal adhesives continued to demonstrate a typical pattern of hydrolytic degradation. This is derived from the water content, an indispensable component for the ionization of acid monomers. In this regard, it is particularly important to respect and, if possible, prolong the evaporation time of the solvent prior to the curing of the adhesive [9,10].

This new class of adhesive was recently described as the “eight-generation” of enamel-dental adhesives, both for their characteristics of use and for the composition (e.g., their content of nanoparticles). In the composition of universal adhesives there can also be nanofillers (10–20 nm), which reinforce the resinous monomers bond to the collagen fibers of the dental tubules, increasing the quality of the hybrid layer with the consequent general improvement of the mechanical strength of the adhesive system. All these chemical features are meant to achieve greater bond strength and clinical durability of the adhesion [4,11–14]. The nanoparticles present inside universal adhesives are involved in the cross-linking during the polymerization phase, reducing polymerization shrinkage. The type of nanometric particles and the method of incorporation within the adhesive affect its viscosity, wettability and penetration ability within the collagen fibers [13].

The versatility of one adhesive technique for multiple substrates is a key feature of universal adhesives. Given their composition and their multipurpose use, and given their use on sclerotic dentin, dentin contaminated by metal oxides and on NCCL (Non-Carious Cervical Lesions) [15,16], it is hypothesized that these adhesives could also operate on pathological substrates, such as on teeth affected by dentinogenesis imperfecta type II (DI-II). Unfortunately, there is limited information in the literature on the adhesion of universal adhesives to DI-II “genetically altered” dentin.

DI-II is an autosomal dominant genetic disorder, generally non-syndromic and limited to dentin. As described by our authors in two previous studies [17,18], the enamel appears normally formed, while the dentin shows an absence of dentinal tubules. Another typical finding is an enamel–dentin junction with a wavy pattern, which is frequently used as a marker for the pathology. In previous works the authors verified the morphology of the hybrid layer of a fifth generation dental-enamel adhesive system on pathological dentin affected by DI-II [17] and on carious dentin affected and contaminated by metal oxides [19]. Since the emerging new universal bonding systems display great performances in restorative dentistry, their use could be helpful even in case of DI-II patients but there is still no evidence of their behavior in this peculiar case. This study is focused on the hybrid layer of two latest generation universal adhesives on dentin comparing sound and DI-II-affected teeth with SEM investigations.

2. Materials and Methods

2.1. Universal Adhesive Systems

Two universal adhesive systems were selected for the experimentation:

All-Bond Universal (Bisco Inc., Schaumburg, IL, USA, batch: 2200001890): single-bottle formulation optimized both for use in total-etch adhesive procedures and for use in self-etch mode (very similar adhesion values in both procedures). It contains MDP monomer, which significantly improves the durability of the seal and adhesion to indirect restorations (zirconia, alumina and metals); it is formulated with a low degree of acidity (pH > 3) with complete compatibility with self and light-curing and dual-curing composites/cements

without the need for an additional activator; the solvent evaporates easily leaving a small residue of water. After polymerization it becomes highly hydrophobic for a long-time seal (reduced water absorption) without the need to add an additional layer of resin. Its low viscosity is meant to allow better penetration in etched surfaces, creating both chemical and mechanical adhesive bonds with the polymerization [5,20].

Universal Bond (Tokuyama Dental, Tokyo, Japan, batch: 103E21): is a two-component (Bottle A + Bottle B) self-curing adhesive system for direct and indirect restorations, usable with selective etching, total etching and self-etching techniques. The adhesive monomer is the 3D-SR (phosphoric acid monomer), which enhances the strength of adhesion to zirconium oxide or metals and gives strong adhesion not only to the dental surface but also to various materials including ceramics, CAD/CAM blocks or metal, in combination with various functional monomers, such as a new silane-based coupling agent or adhesive monomer to precious metals. In addition, a polymerization activator (BoSE technology) is present, obtaining high adhesion strength without the need for photo curing. It improves the bond strength of the polymerizable resinous material (adhesive resin cement, acrylic resin and composite resin) to indirect restorative materials, such as glass ceramic (porcelain), oxide-ceramic (zirconium oxide and alumina), metals (precious and non-precious) and resinous materials, including inorganic filler. It also contains multiple adhesive monomers, in addition to the new 3D-SR monomer, the 6-methacryloyloxy hexyl 2-thiouracil-5-carboxylate (MTU-6) and the γ -methacryloxypropyl trimethoxy silane (γ -MPTES), which are monomers for dental and prosthetic surfaces adhesion; there are also other monomers (HEMA, Bis-GMA and TEGDMA) to form adhesive layers. Acetone, isopropyl alcohol and water are used as solvents and a borate and peroxide-based catalyst as polymerization activators. In order to have a high storage stability, these functional components are kept separated into two bottles, and the adhesion strength is not inferior to traditional light-cured adhesives, thanks to the use of an extremely active polymerization catalyst [5,9].

To evaluate the effectiveness of adhesion on sound and DI-II-affected teeth, four samples, two sound upper third molars and two sound upper third molars affected by DI-II, extracted for periodontal reasons, were collected. All the samples received an X-ray in mesio-distal and vestibular-buccal projections to ensure the absence of carious lesions or other issues.

The samples were cleaned and stored in 0.1% chloramine-T at a temperature of 4 °C for 4 weeks and, subsequently, were stored in saline at 4 °C for another week prior to experimentation.

2.2. Operative Procedures

Each sample was sectioned with a sterile cylindrical diamond bur (coarse grain 2886-314-014, Komet Dental, Lemgo, Germany) with a high-speed handpiece and water spray cooling exposing a flat surface of dentin surrounded by enamel. Prior to performing enamel–dentin adhesion, the surface was then lapped with decreasing grain sandpaper (120, 180, 400 and 600 μm) under abundant cold-water irrigation. Samples were then rinsed with water and gently air-dried for 10 s. Selective etching was performed with 37% orthophosphoric acid (Total Etch, Ivoclar Vivadent Group, Vienna, Austria) for 30 s on enamel and 15 s on dentin. The samples were thoroughly rinsed with water for 10 s and air-dried for another 10 s.

The samples were divided into the following groups, based on the type of adhesive used:

Group 1: Universal Bond (Tokuyama Dental, Tokyo, Japan)—Sound tooth

Group 2: All-Bond Universal (Bisco Inc., Schaumburg, IL, USA)—Sound tooth

Group 3: Universal Bond (Tokuyama Dental, Tokyo, Japan)—Tooth with DI-II

Group 4: All-Bond Universal (Bisco Inc., Schaumburg, IL, USA)—Tooth with DI-II

Groups 1 and 3: following manufacturer indications, two drops of Tokuyama Universal Bond adhesive (one drop bottle A+ one drop bottle B) were mixed in a disposable miniwell and then were applied to the air-dried enamel and dentin surfaces evenly with a microbrush

for 10 s and dried with mild air for 5 s. No light curing is needed since it is a self-curing system [20].

Groups 2 and 4: following manufacturer indications, Bisco All-bond Universal adhesive was applied in two subsequent coats [20] to the air-dried enamel and dentin surfaces scrubbing with a microbrush for 10–15 s per coat, and then dried with mild air for at least 10 s and light-cured for 10 s with LED light-curing (VALO lamp, Ultradent, South Jordan, UT, USA/ λ 385–515 nm) (Table 1).

Table 1. Composition and application of the two universal adhesives systems used.

Adhesive System	Producer	Composition	Application Procedure
Universal Bond 	Tokuyama Dental, Tokyo, Japan	Bottle A: Bis-GMA, HEMA, TEGDMA, MTU-6, phosphoric acid monomer, acetone Bottle B: γ -MPTES, isopropanol alcohol, acetone, water, peroxide, borate catalyst	Adhesive mixed (A+B) was applied to the air-dried enamel and dentin surfaces for 10 s and then dried with mild air for 5 s. No light irradiation was done.
All-Bond Universal 	Bisco Inc., Schaumburg, IL, USA	Bis-GMA, 10-MDP phosphate monomer, HEMA, water, ethanol, initiators	Adhesive was applied two separate coats to the air-dried enamel and dentin surfaces with scrubbing using a microbrush for 10–15 s per coat, and then dried with mild air for at least 10 s. Photocured for 10 s with LED curing light.

After adhesion procedures, each sample was completed with a nanofilled composite resin restoration (Asteria A1B, Tokuyama Dental, Tokyo, Japan, batch: W03010) built by multiple incremental additions simulating a conventional direct restoration and covering the whole adhesive interface. Each incremental layer of composite resin was light-cured for 20 s using the same curing lamp (VALO lamp, Ultradent, UT, USA/ λ 385–515 nm).

All prepared samples were stored in sterile saline solution at room temperature for one week prior to SEM investigations.

After one-week storage time, the samples were longitudinally sectioned into two parts with a 0.3 mm thick diamond disc (Edenta AG, Au, Switzerland) mounted on a slow speed handpiece and then lapped with decreasing grit abrasive paper (200, 400 and 1200 μ m) with copious supply of water.

In order to perform SEM scans, samples were deprived of the roots by dissecting the tooth transversely with a diamond disc (about 0.3 mm thick) (Edenta AG, Au, Switzerland). From each sample, two sections were obtained for the evaluation of the adhesive interface to the SEM. Samples were processed for SEM observation going through dehydration and gold sputtering (Assing Scientific Instruments B7340). The dehydration procedure is necessary for gold sputtering and exposes the sample to mechanical stresses due to different content of water in enamel dentin and resin-based materials. To avoid that it is possible to scan the samples without gold sputtering with a lower image quality in Variable Pressure SEM devices, such as the one that we used but this leads to other artifacts during observation since the SEM will work in partial vacuum and dehydration happens during SEM scan. This strategy would be also less accurate since every observation alters the sample with poor repeatability than it was decided for the standard gold sputtering processing procedure.

All samples were observed using Leo 1450vp Scanning Electron Microscope with SE2 detector, and high SEM magnification was used to observe the hybrid layer formed by the tested adhesive systems (100 \times for the fractures analysis; 3.00K \times for the evaluation of the interfacial bonding layer).

3. Results

The observed SEM-morphology of the hybrid layer on the enamel was similar for the two universal adhesives tested.

On the sound dentin, the morphology and the number of resin tags obtained from the two tested adhesives was comparable to each other and closely resembles the behavior of the fourth and fifth generation etch-and-rinse adhesives with the evidence of resin tags protruding into dentinal tubules (longer tags in the All-Bond Universal sample) (Figure 1).

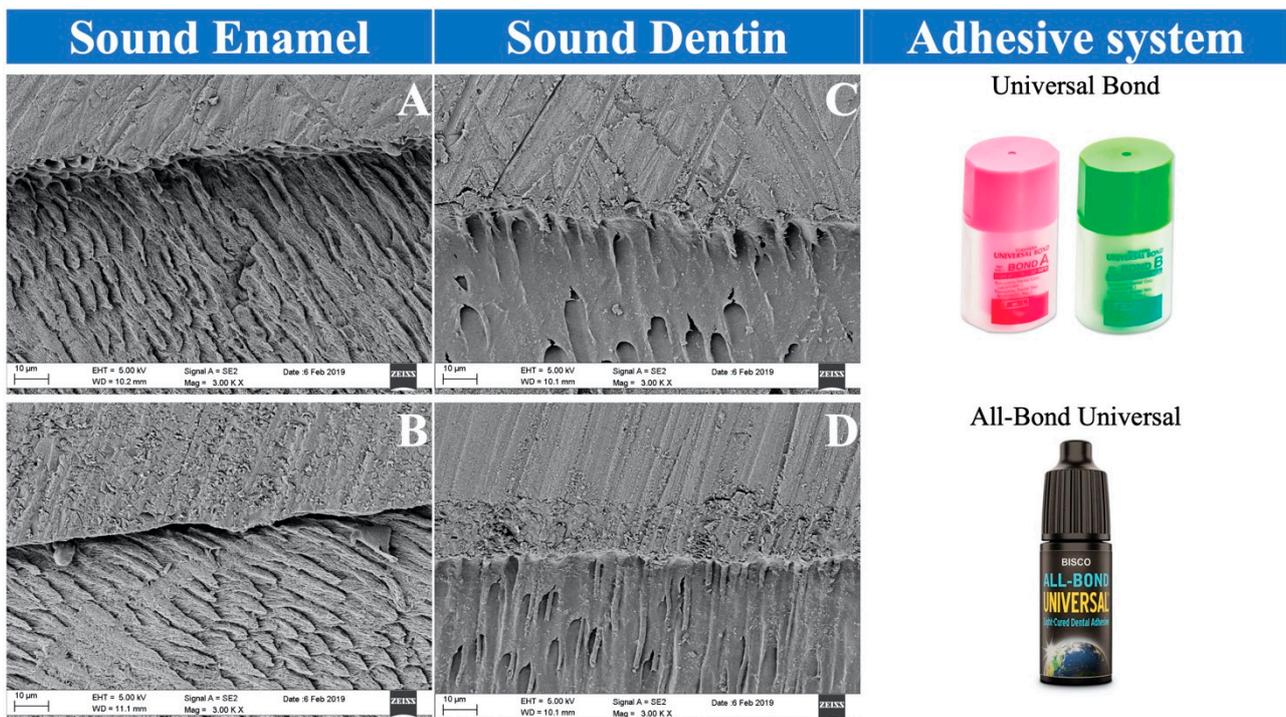


Figure 1. SEM images of interfacial bonding layer for universal adhesives on sound enamel (A,B) and sound dentin (C,D). Images (A,B): composite restoration (upper part) and sound enamel (lower part). Images (C,D): composite restoration (upper part) and sound dentin (lower part).

The SEM analysis of the fractures resulting from the dehydration processes showed that no adhesive detachments occurred on either enamel or sound dentin for both universal adhesives tested, this consideration supports the achievement of good adhesive performance on both normal dental substrates (Figure 2).

SEM photos on enamel of the DI-II-affected tooth confirmed the normal morphology of this substrate and, as in sound teeth samples, the presence of a correct hybrid layer was observed with both the tested adhesive systems. The hybrid layer obtained from both adhesive systems on dentin affected by DI-II, appears adherent to the biological substrate but the absence of resin tags is evident.

The tested universal adhesives on DI-II-affected dentin demonstrated by the formation of an unusual (since the absence of a normal tubular dentinal architecture) yet effective hybrid layer in all the observed sections (Figure 3).

The SEM observation of the fractures, resulting from the dehydration processes of the samples, on a tooth affected by DI-II underlined the quality of the adhesion achieved by the technology of universal adhesives even on an abnormal dentinal substrate totally devoid of dentinal tubules. These results differ from previous works [17], where the behavior of a fifth generation etch-and-rinse adhesive was found to be markedly ineffective on the pathological dentin affected by DI-II (Figure 4). All SEM investigations were performed using Leo 1450vp Scanning Electron Microscope with a magnification of 3.00K \times .

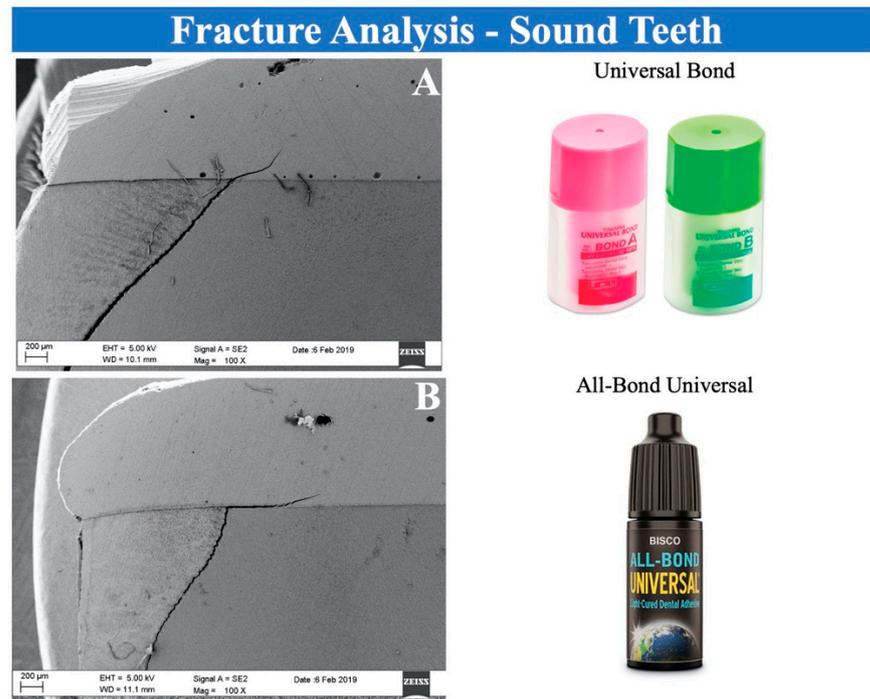


Figure 2. SEM images of fractures in samples with Universal Bond (A) and All-Bond Universal (B) on sound tooth. The fractures depicted occurred during sample processing for SEM due to dehydration; this occurs due to the different content of water between enamel and dentin causing the dentin to shrink and separate from enamel. The way the fracture ends in the composite mass indicates that bonding between dentin and composite is equal or stronger than the natural bonding between enamel and dentin.

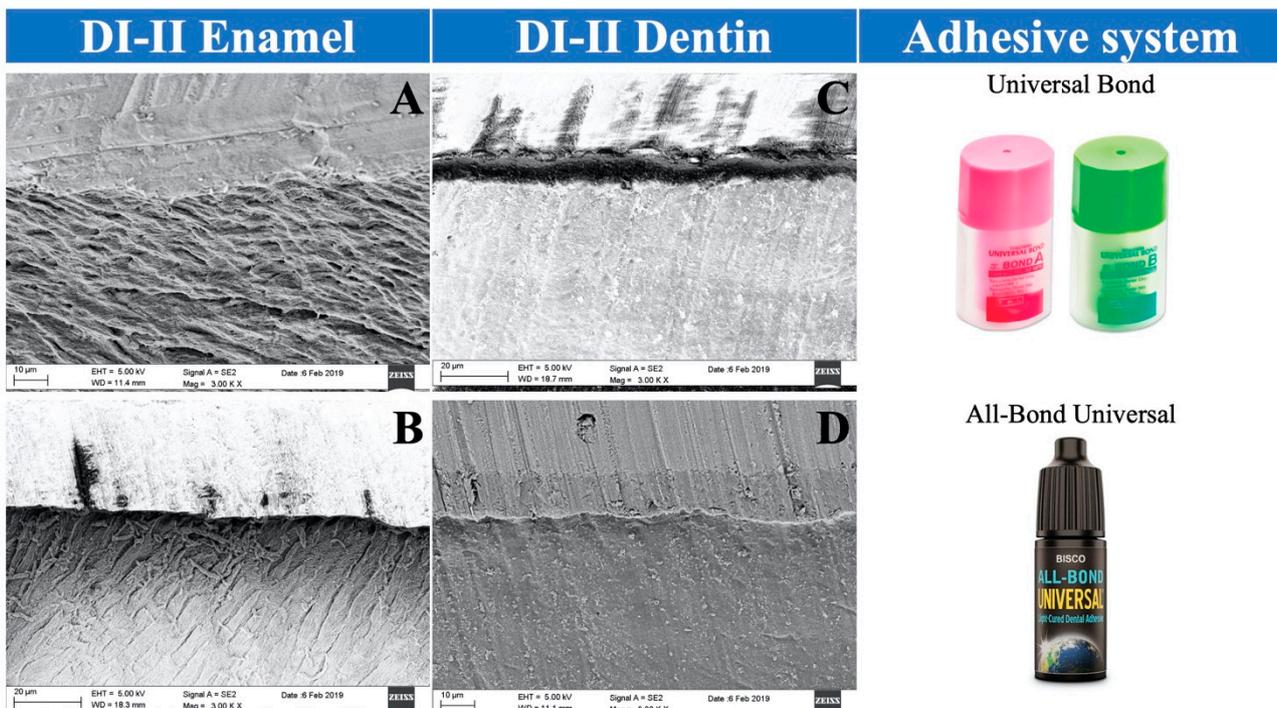


Figure 3. SEM images of interfacial bonding layer for universal adhesives on enamel (A,B) and dentin (C,D) affected by DI-II. Images (A,B): composite restoration (upper part) and sound enamel (lower part). Images (C,D): composite restoration (upper part) and DI-II-affected dentin (lower part).

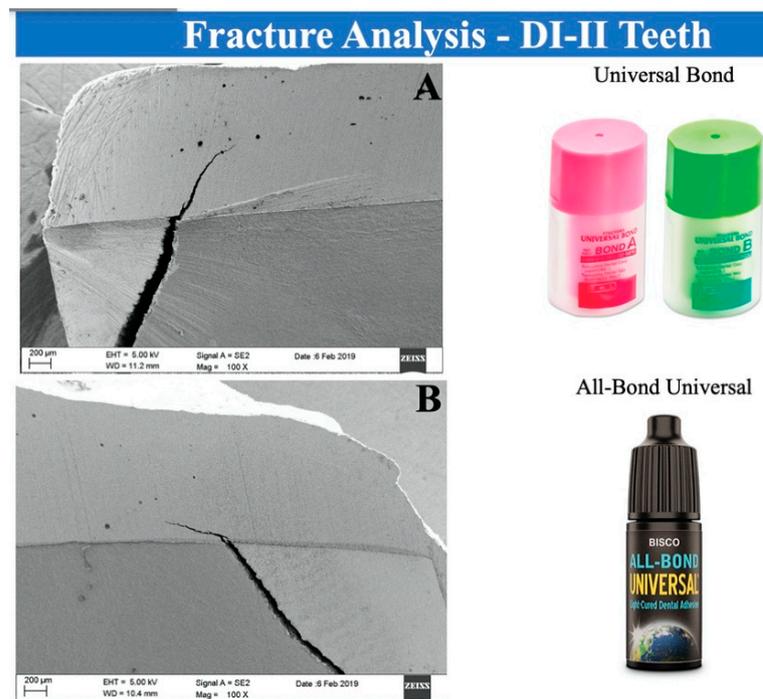


Figure 4. SEM images of fractures in samples with Universal Bond (A) and All-Bond Universal (B) on a tooth affected by DI-II. The fractures depicted occurred during sample processing for SEM due to dehydration; this occurs due to the different content of water between enamel and dentin, causing the dentin to shrink and separate from enamel. The way the fracture ends in the composite mass, tells that bonding between dentin and composite is equal or stronger than the natural bonding between enamel and dentin.

4. Discussion

The rapid spread of the latest generation universal adhesives and their versatility had prompted us to test them on sound and on DI-II-affected teeth. Only in recent years the literature started to talk about these new adhesive systems. Literature on these adhesives is principally oriented to sound teeth, dentin contaminated by metal oxides, NCCL and demineralized enamel [20], but there is no research on adhesion to DI-II-affected teeth.

These adhesives showed similar SEM appearance to fourth and fifth generation adhesives on sound dentin and enamel with a thin interface in enamel and a visible hybrid layer in dentin with formation of resin tags in dentinal tubules. The advantage of the newer universal bonding adhesives is that they can be used in both self-etch and total-etch mode. The behavior of both tested adhesives was found to be effective and similar on both sound enamel (Figure 1A,B) and on enamel affected by DI-II (Figure 3A,B). This was expected since the structure of the enamel affected by DI-II, as previously discussed [18], is identical in quality and structure to the sound one. Even if universal adhesives are capable of adhesion on enamel in self etch mode, the literature agrees that a dedicated etch and rinse step leads to better adhesive performance on the highly mineralized enamel substrate. The choice of using these universal adhesives in an etch-and-rinse mode prepared the enamel surface to be properly wetted by the adhesive helping to achieve chemical and micromechanical bond in enamel.

On the other end, on the dentinal substrate, the etch-and-rinse step prior to adhesive application is not mandatory since the chemical characteristics of the universal bonding systems is capable to deal with the leftover smear layer creating in this case a thicker but less penetrating hybrid layer. Adhesion to dentin is highly affected by its heterogeneity in microanatomy (number and diameter of tubules) and mineralization, leading to a vast variability of bonding strength even in the same dentin of the same tooth [19]. Differences between adhesive interface in sound and DI-II-affected dentin was expected and to reduce

the number of variables the universal adhesives were used removing the smear layer before adhesion with an etch and rinse technique [9].

The etch and rinse procedure on dentin leads to the increased availability of dentinal tubules that can be infiltrated by the adhesive. The reduction of the presence of water that wets the surface, due to the drying process, increases the quantity of infiltrating resin and therefore the quality of the resin tags. The morphology of the hybrid layer on dentin of the two tested adhesives appears similar to that of the self-etch adhesives and the fourth and fifth generation adhesives reported in the literature (Figure 1C,D) [9]. The morphology of the tested adhesives on DE-II-affected dentin (Figure 3C,D) seems to confirm the possibility of using them in selective etch-and-rinse mode on dentin as was made in our previous works with a fifth-generation adhesive [17,18]. The adhesive failure found on the DI-II-affected samples treated with a fifth-generation adhesive in a previous work [17] was not found in the samples treated with the two universal nanofilled adhesives tested in this study (Figure 3C,D). The quality of the hybrid layer on DI-II-affected dentin, even if in total absence of resin tags, can be appreciated in Figures 2 and 4. In all samples (sound and DI-II-affected teeth), the dehydration of the samples, due to processing for SEM observation, produced fractures through the enamel–dentin junction. The fractures intercept the composite reconstruction without debonding the above restoration from either the enamel or the dentin. This behavior was equivalent to that observed for samples on sound teeth (Figure 2), where similar features were observed and no separation of the restoration from either sound dentin or sound enamel was present.

The SEM appearance of the hybrid layer on sound dentin confirmed the great versatility of All-Bond Universal and Universal Bond adhesives in managing adhesion on different dental substrates. The quality of the hybrid layer observed on DI-II-affected enamel and dentin by these adhesives suggest their choice in these puzzling clinical situations. The possibility of using such universal nanofilled adhesives is an advantage for direct techniques but especially for indirect techniques [21]. The use of adhesive composite indirect restorations in DI affected patients was documented in previous works that presented the esthetic and conservative full mouth rehabilitation in a DI-type II-affected patient [17]. The thirteen year follow-up of the same case showed optimal long-term stability of the restorative technique using two steps adhesive systems and indirect restorations cemented with light cured composite restorative materials [18]. SEM images of newer “Universal” bonding systems appear to be less sensitive to substrate variability than the previous generation adhesives, thus also suggesting their use for adhesion in the dentin of DI affected patients.

These adhesive systems allow adhesion on dental substrates and on virtually all indirect restorative material [22].

5. Conclusions

The ease of use of these universal nanofilled adhesives is a great improvement on the management of many common clinical situations, such as the presence of secondary atubular dentin beside sound dentin in deep restorative preparations, both in direct and indirect restorative procedures, also reducing operator-dependent procedural mistakes due to more complex or multiple steps techniques.

Like any other adhesive system, a main concern is the stability of the hybrid layer ageing in time. Further clinical studies are necessary to investigate in depth the long-term stability of the initial adhesion strength of universal adhesives on pathologically or genetically altered dentinal substrates.

Limitations of the study: An important limitation of this in vitro pilot study is the small number of samples and of different adhesive systems tested. Obviously, this is related to the difficulty of collecting teeth from DI-II-affected patients (incidence 1/8000 [23,24]), leading to a very small sample size; to address this issue, future multicentric studies are required.

Author Contributions: Conceptualization, G.G., A.L., V.C., V.D.T. and L.M.; methodology, A.L., V.D.T., L.M., G.G. and V.C.; software, G.G. and V.D.T.; validation, G.G., A.L. and V.C.; formal analysis, A.L.; investigation, V.D.T. and A.L.; resources, V.C.; data curation, V.D.T.; writing—original draft preparation, V.D.T. and A.L.; writing—review and editing, G.G., A.L., V.D.T.; visualization, V.C.; supervision, V.C.; project administration, V.C.; funding acquisition, V.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study.

Conflicts of Interest: The authors declare no conflict of interest.

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