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Morphological comparative study between a fifth-generation adhesive system and a universal system in total-etch mode applied at the cervical region

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Abstract

For both direct and indirect restorations at the cervical level, the preparation aims to ensure only enamel bonding, due to its proven performance over time. This study sought to evaluate the adhesion to the cervical tooth structure. The aim of the study was to morpho-structurally compare a two-step adhesive system with a universal system in total-etch mode, starting from the hypothesis of the presence of only enamel as the bonding substrate at the level of cavities prepared to the standard depth of 0.4 mm, located 2 mm coronal to the cementoenamel junction. Each adhesive system was assigned a batch of three tooth samples with standard prepared and filled cavities. From each tooth sample, three sections were cut in bucco-lingual direction at the level of the fillings. The sections were prepared for examination by scanning electron microscopy (SEM), using a specific protocol for demineralization and deproteinization. The fifth generation system presented well-represented enamel micro-retentions. Hiatuses were observed at the level of the dentin hybrid layer, despite the formation of intra-tubular resin plugs. The universal system presented less evident micro-retentions on enamel. The thickness of the adhesive layer of the universal system presented higher values compared to the fifth generation system, for both hard dental tissues. Minimal preparations may present dentin exposure, depending on the location of the cervical termination. Acid etching before applying the universal system increased its performance. In the case of enamel, the fifth generation system shows a more favorable adhesion, due to micro-mechanical retention.

Keywords: dental adhesion, minimally invasive dentistry, enamel, dentin, cervical region.

Introduction

The progress in restorative dentistry is largely due to the evolution of dental materials that adhere to the dental substrate. The development of clinically effective adhesive systems on hard dental substrates allows for the abandonment of the need to sacrifice healthy tooth structure through the classical configuration of the preparation that provides macro-mechanical retention to accommodate the non-adherent restorative material [1]. Fundamental to the adhesive technique is the achievement of a bond between the hard dental substrate and the adhesive system, based on micro-mechanical retention and chemically interacting resin monomers [2, 3].

The cervical area is of interest in restorative dentistry in the case of bonding of direct restorations [4] and indirect ones [5]. This topic is also of interest in the field of periodontology, as gingival recessions reveal the root zone [6], and the patient complains of sensitivity and aesthetic disturbance, requesting covering the defect. In orthodontics, the cervical region is closely related to the placement and maintenance of fixed orthodontic appliances, while the surrounding gingival tissues may undergo significant morphological and inflammatory changes during treatment [7]. Therefore, in such clinical contexts, it is important for the practitioner to know the intrinsic properties of the substrate to which the adhesive bonding of the restoration will be performed, since at the cervical-radicular level there are multiple forms of hard dental substance: enamel, the cementoenamel junction (CEJ), cervical dentin, respectively radicular dentin. It is confirmed that adhesive failure is most disastrous in restorations with margins located below CEJ [8].

The dental adhesion is a micromechanical process of communion between the dental substrate and the adhesive system, achieved by interposing the adhesive at the irregularities of the dental substrate – in the case of enamel, through acid-etched enamel prisms, and in the case of dentin, through the collagenous structure. The adhesive forms chemical bonds with both the inorganic substrate and the organic substrate [9]. The introduction of acid etching on dentin and the total-etch technique appeared with the studies

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of Fusayama *et al.* [10], who advocated for the difficulty of acid etching only the enamel substrate without touching the neighboring dentin, the etching achieving demineralization of dentin and smear layer, which reveals collagen fibers capable of being impregnated with resin. To maintain the permeable collagen skeleton, the primer is used, which is a hydrophilic resin in a solvent such as water, acetone or alcohol, whose role is to wet the collagen network, due to its hydrophilicity [11].

Ideally, the adhesive systems should have similar bond strengths to enamel and dentin to avoid debonding of the restorative composite from the less adherent dental substrate during light curing [12]. The most predictable and stable long-term adhesive system remains the three-step total-etch system, in which the acid/conditioner, primer, and adhesive resin are applied separately [13]. The fifth generation represents a simplification of the latter, by combining the primer and the bonding agent in the same bottle [14]. The seventh adhesive system generation ("one bottle" system) is very attractive to clinicians, by using a single bottle that encompasses all stages in one simplified step.

Aim

The general purpose of the study was the comparative analysis, by scanning electron microscopy (SEM), of two adhesive systems (total-etch and one-bottle) applied in cervical tooth preparations, from a morphological qualitative point of view.

Materials and Methods

For this research, an *in vitro* experimental study was conducted with extracted human teeth. Six teeth were selected, divided into two sets (for each adhesive system), respectively one upper incisor, premolar and molar for the application of one of the selected adhesive systems. To be included in the study, they had to be free from any restoration or cervical lesion on the buccal surface on the portion where the cavity would be prepared, and the contour of the CEJ had to be clear, as a reference for the placement of the cavity in the vertical direction. Permanent human teeth were taken and maintained in accordance with the ISO/TS 11405:2015(E) standard (testing of adhesion to dental structures). Immediately after extraction, the teeth were stored in aqueous solution with 0.5% Chloramine (Figure 1).

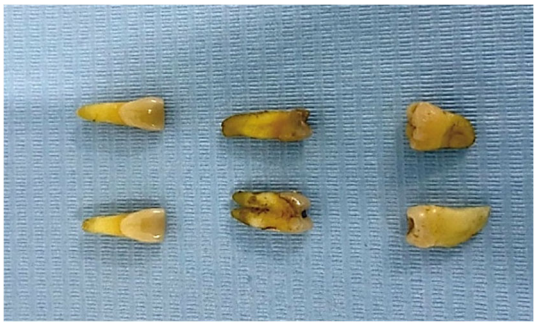

Figure 1 – *Selection of teeth to be prepared, after debridement of the coronal-radicular surfaces.*

Before the actual cavity preparation, the outline of the CEJ traced on each tooth with a fine marker as a guide for orienting the height of the cavities (Figure 2, A–C).

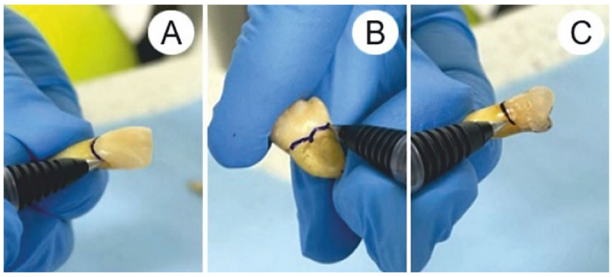

Figure 2 – *(A–C) Tracing the outline of the CEJ with a fine marker (the bottom of the cavity will be located 2 mm coronal of it). CEJ: Cementoenamel junction.*

Using a 0.8 mm diameter spherical diamond stone with a blue ring mounted on the turbine and under water cooling, standardized cavities were made in the cervical area of the buccal surface 2 mm coronal to the CEJ, with a length of 6 mm parallel to the collar line, a depth of 0.4 mm and a width of 0.8 mm. The 6 mm length of the cavity allowed the creation of multiple sections (more precisely, three sections) in the buccal-lingual direction of the sample tooth (Figure 3, A–C).

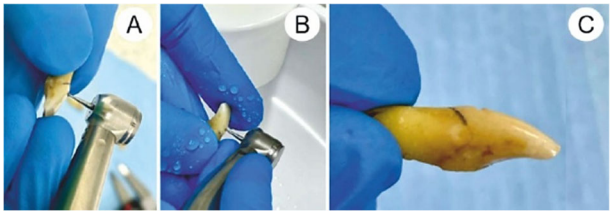

Figure 3 – *Moments during cavity preparation: (A and B) The position of the active head of the diamond bur in relation to the CEJ, respectively the geometry of the resulting cavity; (C) Width of 0.8 mm (equal to the diameter of the spherical stone) and depth of 0.4 mm (equal to half its diameter).*

The teeth were divided into two sets (set "5", set "U"), so that each set included an incisor (I), premolar (P) and molar (M). Subsequently, each tooth will be sectioned into three samples for microscopic analysis (sample names will be, for example, 5-I-1, U-P-2, etc.). The cavities were assigned a treatment represented by the application of the adhesive system, in this case the teeth were divided into two batches, each corresponding to a distinct adhesive system: the fifth generation adhesive system *Adper™ Single Bond 2 Adhesive* (*3M Espe*) for the "5" group and the universal adhesive system applied in the total-etch mode *Single Bond Universal Adhesive* (*3M Espe*) for the "U" group. The adhesive protocol followed manufacturer's recommendations: acid etching with 37% phosphoric acid (*Bisco*) for 30 seconds, washing with water for 10 seconds and drying the etched cavity with a dry cotton pellet, application of the adhesive system by rubbing for 15 seconds in the case of *Adper™ Single Bond 2 Adhesive*, respectively 20 seconds for *Single Bond Universal Adhesive* (the slightly longer application time for this system is due to the presence of 10-methacryloyloxydecyl dihydrogen phosphate (MDP) monomers, which require sufficient time for chemical interaction with the calcium of dental hydroxyapatite crystals [15]), light drying with air spray for five seconds, light curing for 10 seconds. Subsequently, the cavity was filled with a flowable diacrylic composite resin (*Gradia Direct LoFlo/GC*) of shade A1 (due to the color contrast allowing easy identification of the filling during the sample

processing process) by applying a single layer, light-cured for 40 seconds and marginally adapted with a periodontal probe. The teeth were kept in two separate trays depending on the adhesive system used and preserved in water until their processing for microscopic examination.

For sample preparation for SEM, each tooth was clamped in a metal clip for stability and then inserted into a silicone well for cold embedding (Figure 4, A and B).

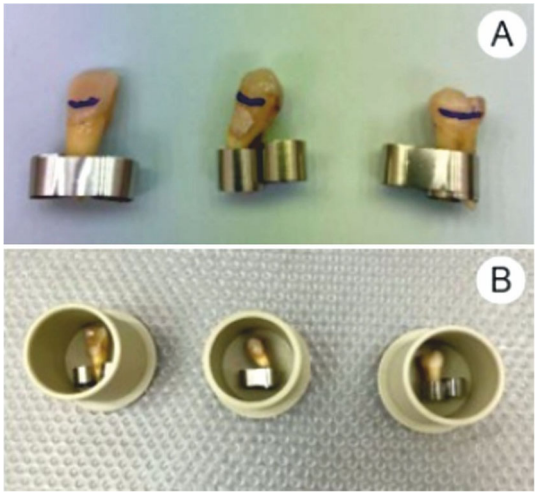

Figure 4 – ***(A) Fixing each tooth in a metal clip for its positional stability in the well with investment resin; (B) Carefully inserting the fixed teeth into the well, exposing the surface opposite to the one with the filling of interest.***

For cold embedding, a rapid-setting kit consisting of a dibenzoyl peroxide-based powder and a *p*-toluidine-isobutyl-dimethacrylate-based liquid was used, in a 2:1 ratio (*Sampl Kwick/Buehler*, Lake Bluff, IL, USA). Figure 5 shows the final appearance of the embedded trial teeth, with the buccal surface exposed with the filling highlighted with the marker.

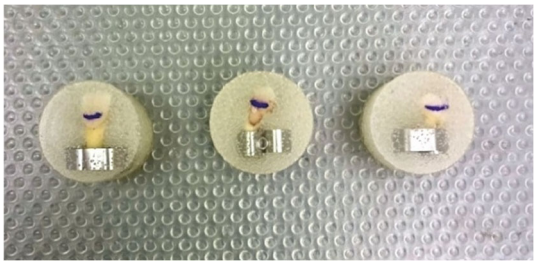

Figure 5 – ***Resin-embedded sample teeth.***

After the tooth embedding stage, sections were made using a *DIMOS 19-100* precision wet diamond cutting disc mounted on a *Metkon MICRACUT 202* automatic cutting machine (*Metkon Instruments Inc.*, Bursa, Turkey), using a low velocity (Figure 6A). Due to the standard width of the fillings of 6 mm, subsequently colored with the marker, it was allowed to obtain three slices from each sample tooth (Figure 6B). Each slice was approximately 1.7–2 mm thick (considering the 0.4 mm disc thickness for each cut).

After being cut, all slices were carefully cleaned in deionized water with ultrasound for three minutes, rinsed with ethyl alcohol and air dried. This was followed by the process of fixing with epoxy adhesive (*Loctite EA E04/ Henkel*, Italy) on aluminum buttons of each sample separately, resulting in a total of 18 samples. The role of fixing the samples on the buttons is to facilitate their handling during the following steps, including the actual SEM examination. The next step was the grinding and polishing of each sample, which was carried out using a *Metkon DIGIPREP Accura* automatic grinding and polishing machine (*Metkon Instruments Inc.*, Bursa, Turkey). A 20 µm *XDMB diamond disc* (*NXMET*, Echirolles, France) was used for the grinding operation under continuous water irrigation. The polishing operation consisted of several steps, using the same machine as in the previous operation. For this purpose, special soft textile discs (*VerduTex, MicroCloth/ Buehler*, Lake Bluff, IL, USA) were used, impregnated with polycrystalline diamond suspensions of 3 µm and then 0.25 µm (*MetaDi Supreme 73 Diamond/Buehler*, Lake Bluff, IL, USA). Finally, a super-polishing step followed, using a creamy suspension of 0.04 µm *XALO aluminum oxide* (*NXMET*, Echirolles, France) on a *MicroCloth/Buehler* textile disc. Between the grinding and polishing steps, to avoid cross-contamination, the samples were carefully cleaned in distilled water with ultrasound for three minutes, rinsed with ethanol and dried in a stream of air.

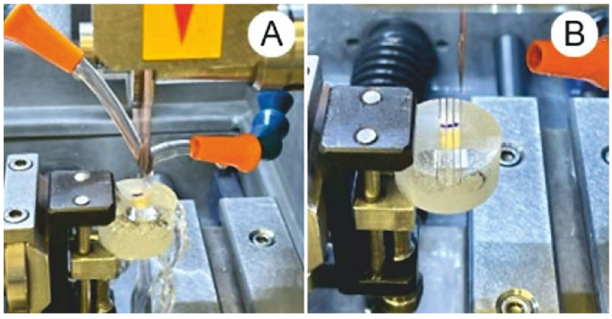

Figure 6 – ***The stage of cutting teeth into sections, (A) before and (B) after.***

To highlight the formation of intratubular resin plugs in dentin, the samples were etched with 15% hydrochloric acid for 30 seconds and then deproteinized by immersion in 3% sodium hypochlorite (NaOCl) solution for 30 seconds. Between the two stages of chemical treatment, possible residues were removed by rinsing with distilled water with ultrasound for 15 seconds, then with ethanol and drying in a stream of air.

To make the samples electroconductive, a specific aspect of an SEM examination, their surface was treated by gold nanocoating (in a 5 nm layer), using a *Quorum Q150R ES* Sputter coater (*Quorum Technologies Ltd.*, East Sussex, UK) (Figure 7A), finally the samples having the appearance according to Figure 7B.

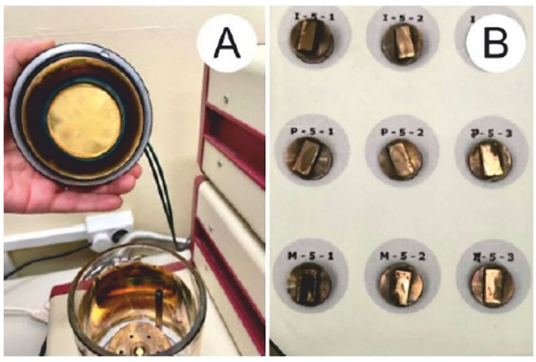

Figure 7 – ***(A) Gold nano-coating machine; (B) Appearance of treated samples coated with gold nano-coating.***

Finally, the prepared samples were subjected to SEM examination, being placed one by one in the vacuum chamber of the *TESCAN Vega IV LMU* microscope (Brno, Czech Republic). Thus, it was possible to analyze for each section the dental substrate and the associated adhesive interface, with the recording of captures at variable magnifications.

Results

For comparative analysis, captures of the morphology of enamel and dentin were made after grinding and polishing stage (Figure 8, a and b) and after the aforementioned chemical treatment (Figure 9, a and b; Figure 10, a and b).

If the interprismatic component is intact in the untreated enamel, it will be affected during the demineralization and deproteinization treatment, since the interprismatic substance and the prismatic sheaths are hypocalcified and rich in organic matter. Thus, in Figure 9b the centers of the prisms with a fish scale appearance are visible. In turn, they are formed by acicular hydroxyapatite crystals arranged in bundles. At the dentinal level (Figure 10, a and b), the treatment applied demineralizes the hypermineralized component of the peritubular dentin, which surrounds the tubule orifices, and the intercanalicular dentin acquires an amorphous appearance. The lumen of the tubules is exposed to its contents.

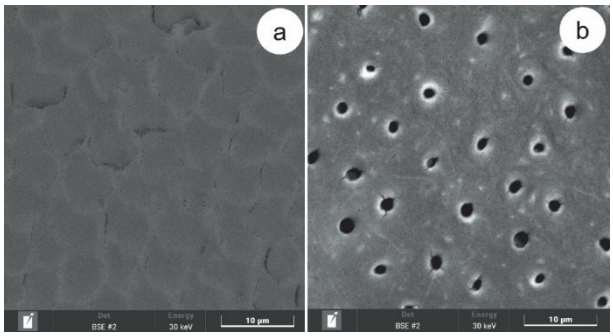

Figure 8 – ***(a) SEM image characteristic of enamel (not chemically treated by acid etching) (×5000); (b) SEM image characteristic of dentin (not chemically treated by acid etching) (×5000). SEM: Scanning electron microscopy.***

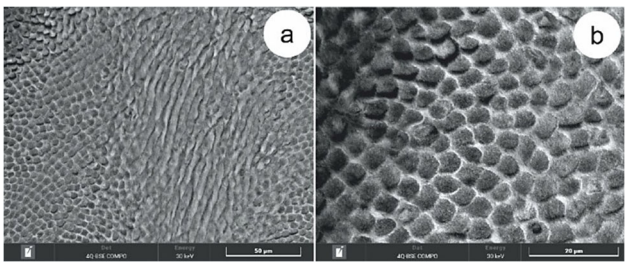

Figure 9 – ***SEM images characteristic of chemically treated enamel (acid etching): (a) Enamel sectioned longitudinally and transversely (×1000); (b) Detail of prisms sectioned transversely (×3000).***

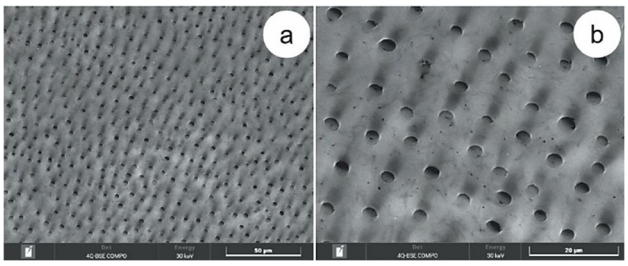

Figure 10 – ***SEM images characteristic of chemically treated dentin (acid etching) (×1000); a – overall view of dentin with tubular structure; b – detail, (×3000).***

Subsequently, the enamel–adhesive and dentin–adhesive interfaces were investigated at the sample level, for each group depending on the adhesive system.

"5" set

Enamel–adhesive–composite interface

At this level, the formation of an adhesive film of homogeneous and compact thickness is observed, which creates resin plugs with the enamel prisms through micro-mechanical retention (Figure 11, a–d). This aspect represents the classical enamel bonding pattern, by forming, from the outside to the inside, a compact sealing layer, resin plugs through prismatic dissolution and coupling with them, and in their depth the prism crystals merge with resin micro-digitations. In Figure 11b, longitudinally cut prismatic cords are observed, which at the adhesive interface present themselves with their ends in the shape of fish scales, which ensure the micro-mechanical character of the adhesion.

Dentin–adhesive–composite interface

In Figure 12 (a–d), the dentinal adhesion is shown, the dentin being captured in longitudinal section (by the presence of dentinal tubules with longitudinal and parallel paths between them), at the interface with the hybrid layer the tubular orifices are visible. The hybrid layer is detached from the dentin and glued to the restoration composite, being arranged in a homogeneous layer in thickness. However, despite the hiatus between the adhesive and the dentin, intratubular resin plugs were produced, which entered deeply into the tubules, but separated from the hybrid layer. In detail in Figure 12d there are good digitations of the plugs that penetrated the tubular lateral ramifications, the resin having a high penetrability at this level. Therefore, the adhesion to the dentin had in this case both a chemical and micro-mechanical basis, by the presence of the dentinal tubules that exposed their orifices to the interface.

"U" set

Enamel–adhesive–composite interface

In Figure 13 (a–d), the enamel is sectioned obliquely, being very clearly visible the parallel prisms with longitudinal path and arranged in layers (Figure 13b). At the level of the hybrid layer these create the micro-retentions less represented, with irregular appearance. In contrast, the hybrid layer is consistent and of increased thickness.

Dentin–adhesive–composite interface

Figure 14 (a–d), illustrates the adhesion to dentin. The hybrid layer is of increased thickness and seals the dentinal wound, without interfacial dehiscence. In Figure 14 (c and d), the micro-mechanical connection with the dentinal substrate is better visualized by creating intracanalicular resin branches.

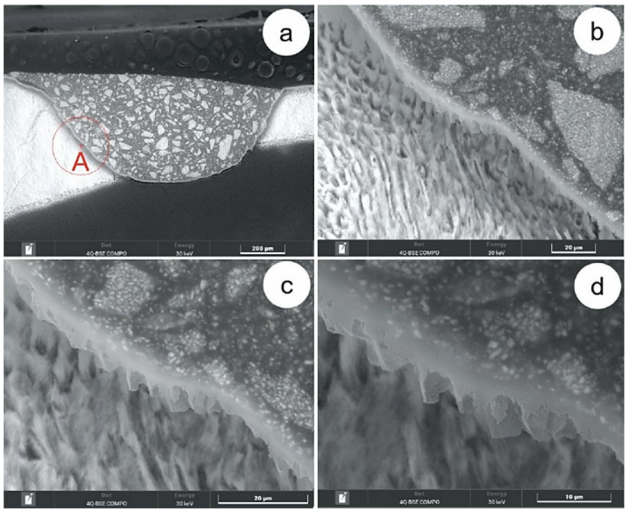

Figure 11 – ***SEM images characteristic of the premolar – fifth generation adhesive (5-P) system: (a) 5-P system (×150); (b) detail of the enamel–adhesive–composite interface (×1500); (c) Detail of the enamel–adhesive–composite interface (×3000); (d) Detail of the enamel–adhesive–composite interface (×5000).***

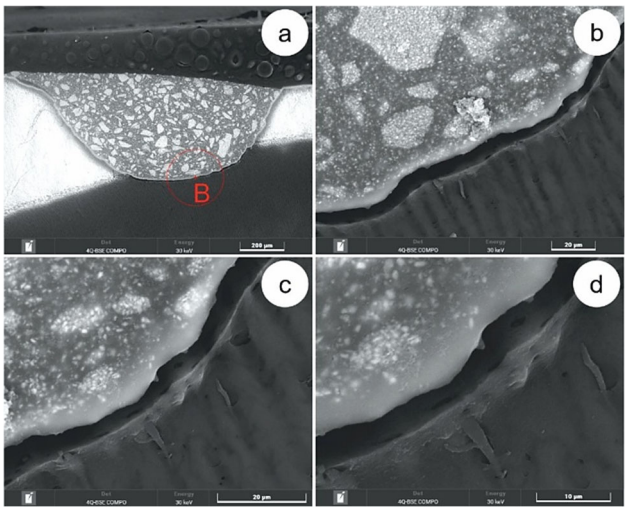

Figure 12 – ***SEM images characteristic of the premolar – fifth generation adhesive (5-P) system: (a) 5-P system (×150); (b) Detail of the dentin–hybrid layer–composite interface (×1500); (c) Detail of the dentin–hybrid layer–composite interface (×3000); (d) Detail of the dentin–hybrid layer–composite interface (×5000).***

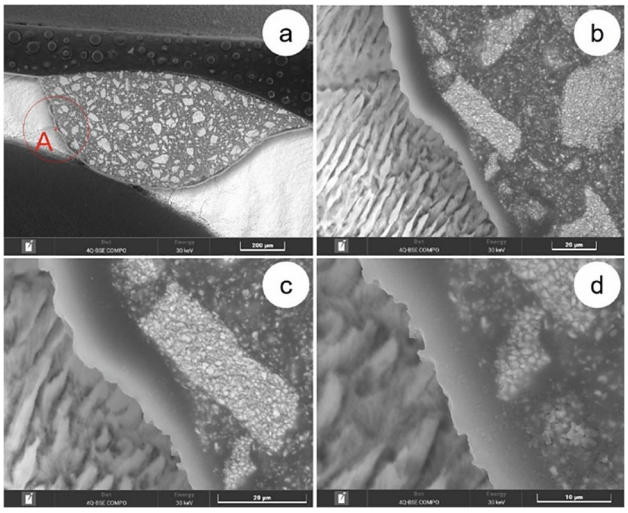

Figure 13 – ***SEM images characteristic of the premolar – universal adhesive (U-P) system: (a) U-P system (×150); (b) Detail of the enamel–adhesive–composite interface (×1500); (c) Detail of the enamel–adhesive–composite interface (×3000); (d) Detail of the enamel–adhesive–composite interface (×5000).***

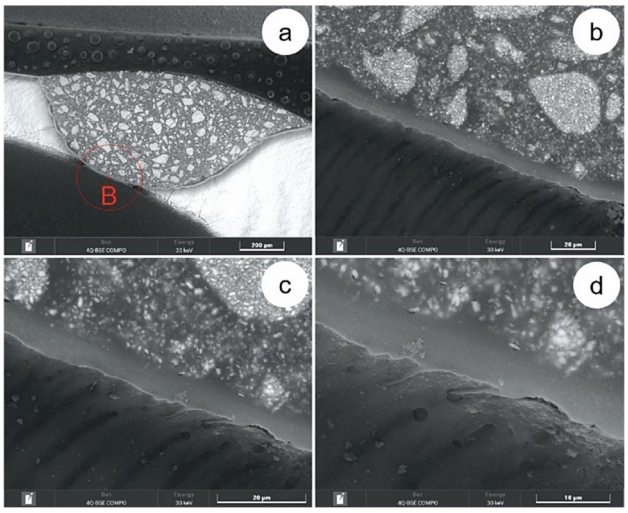

Figure 14 – ***SEM images characteristic of the premolar – universal adhesive (U-P) system: (a) U-P system (×150); (b) Detail of the dentin–hybrid layer–composite interface (×1500); (c) Detail of the dentin–hybrid layer–composite interface (×3000); (d) Detail of the dentin–hybrid layer–composite interface (×5000).***

Discussions

The present study of adhesion in the cervical area has focused on the value of 0.4 mm of the standardized cavity depth, since this preparation depth allows, in the case of indirect restorations, the creation of margins for monolithic restorations (made of feldspathic ceramic or zirconium dioxide, respectively metal). As Magne & Belser state in the book "*Biomimetic restorative dentistry*" [16] about ceramic restorations and the design of the preparation for them, the 0.3–0.4 mm deep chamfer termination at the supragingival level ensures positional stability of the restoration margin (good support of the ceramic and optimal marginal closure, compared to the feather-edge termination or the "no-prep" approach), chameleonic effect and invisible aspect of the transition between tooth and restoration [17, 18]. By placing the margin 2 mm coronal to the CEJ, it is most likely juxtagingival or slightly supragingival, but by using glass-ceramics, the margin will be very well integrated aesthetically and, in addition, by being located away from the bottom of the gingival sulcus, it will protect the superficial periodontium. In addition, biofilm accumulation on ceramics is less compared to other restorative materials, such as metal alloys, composite diacrylic resins or even with the tooth's own hard tissues [19, 20].

The location of the edges of a preparation 2 mm coronal to the CEJ, with a depth of 0.4 mm, does not completely avoid the risk of penetration into the cervical dentin [21, 22]. The sample teeth had all the lateral walls in enamel, but in certain teeth (premolars of both groups and one of the

incisors) the bottom of the cavity exceeded the enamel–dentin junction. Therefore, to avoid this inconvenience, it is necessary to place the edge of the same depth slightly more coronal, respectively to reduce the depth of the preparation located at the analyzed level on the longitudinal axis to 0.3 mm. Otherwise, the possibility of dentin exposure must be taken into account and a therapeutic strategy must be applied in this regard, by obtaining an optimal dentin adhesion with the help of certain adhesive systems used according to the clinical situation. For a clinically acceptable bond of the glass-ceramic restoration, the following criteria must be met: at least 50% of the surface is enamel, and at least 70% of the cervical end is enamel [23–25]. Additionally, the way the enamel is presented on the section in relation to the adhesive interface is very important: longitudinally cut prisms provide a much weaker adhesion than transversely cut ones, exposing the prismatic ends to the bond with the adhesive system [25, 26]. At the dentinal level, the morphology of the hybrid layer and the penetration of adhesive systems into the dentinal tubules are also strongly influenced by the characteristics of the substrate and the adhesive strategy employed [27].

In our study, enamel adhesion by both types of adhesive systems was favorable, with acid etching ensuring micro-mechanical retention. The resin plugs created by inter-connecting with the enamel prisms were better represented in the case of the fifth generation system than in the universal one. This fact certifies the adhesion capacity of the universal system, which relies predominantly on chemical mechanisms and to a lesser extent on micro-mechanical ones [28–31]. In the morphological analysis of the fifth generation system samples at the dentin level, an inferior quality of the dentin wound was noted, compared to the universal system. The detachment of the adhesive layer from the exposed dentin was noted in our study, despite the formation of obvious intratubular resin plugs, which ensure the micro-mechanical nature of the adhesion mechanism through this system. In the universal system, the dentinal surface remains tightly sealed by the adhesive film through the formation of the hybrid layer, due to the participation of the chemical adhesion mechanism [32, 33]. Compared to *Adper™ Single Bond 2 Adhesive* (*3M Espe*), *Single Bond Universal Adhesive* (*3M Espe*) has 10-MDP acidic monomers that allow its use in self-etch mode, namely the acidic conditioning of the dentin that produces limited decalcification, thus being considered the most optimal method of treating the dentinal surface through moderate acidity and chemical interaction with hydroxyapatite crystals, resulting in stable and insoluble salts of phosphate and calcium carboxylate [34, 35].

SEM remains a valuable method for evaluating the morphology of the adhesive interface and the interaction between resin materials and dental substrates [36, 37]. In our study what was observed after the demineralization and deproteinization treatment of the samples in the form of a thin hyper-dense layer at the interface between the adhesive and dentin is identified as hybrid dentinal detritus was also described by Takamizawa *et al.* [38] in the SEM analysis of various universal systems, including the *Single Bond Universal Adhesive* (*3M Espe*) taken in the present research. The presence of this structure is likely to certify the chemical interaction at the adhesive–dentin interface [38]. The use of the universal system in the total-etch mode also allowed the formation of intratubular resin plugs, including at the level of their branches. The chemical mechanism of adhesion between the resin monomers and the pericanalicular dentin probably contributed to their production [39].

Conclusions

Cervical cavities made to a standard depth of 0.4 mm presented both enamel and dentin as adhesion substrates in this study. Reductions greater than 0.4 mm near the gingiva will inevitably lead to denudation of cervical dentin, so it is crucial that the preparation design for indirect restorations anticipates the minimum space requirement for the restorative material, one of the prerequisites for success being adhesion to enamel surfaces. Therefore, in the context of restorative dental therapy, the possibility of requiring bonding also in dentin, a dental tissue that responds less predictably to adhesive techniques than enamel, should be considered.

Adhesive systems with separate acid etching, both on enamel and dentin, present different behaviors and morphologies depending on their composition and the properties of the adhesion substrate. Thus, depending on the type of dental hard tissue, certain types of adhesive systems may be preferred. The present study, which compared a representative fifth generation system with a universal one in total-etch mode, suggested that fifth generation systems present an adequate behavior on enamel (through a strong micro-mechanical retention given by the resin plugs), but at the dentinal level they appear weaker than universal systems (although since they also form intra-tubular resin plugs, the sealing effect of the dentinal adhesion surface is lost), most likely due to the chemical adhesion that universal systems achieve with dentin through the 10-MDP functional monomers.

Conflict of interests

The authors declare that they have no conflict of interests.

Authors' contribution

All authors of this research paper have directly participated in the planning, execution, or analysis of this study, and also all authors of this paper have read and approved the final version submitted and share first authorship.

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