Analysis of the bonding interface in human dentin of two adhesive systems with and without the use of chlorhexdine in the cementation of ceramic restorations

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Analysis of the bonding interface in human dentin of two adhesive systems with and without the use of chlorhexidine in the cementation of ceramic restorations

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Abstract

The degradation of the adhesive interface of indirect ceramic and dentin restorations results from the interaction of several factors, including an enzyme called metalloproteinase. The objective of this study was to evaluate the interface of ceramic-dentin bonding by varying the use of chlorhexidine and of the conventional and self-etching adhesive systems. The dentin surface was divided into four areas: mesiobuccal, distobuccal, mesiolingual, and distolingual, forming four groups (n=4): G1 (MB) SB + SCL; G2 (DB) SB + CCL; G3 (ML) EO + SCL; G4 (DL) EO + CCL. Two mesiodistal cuts were made, thus, exposing the dentin interface-adhesive systems. The results showed that the use of chlorhexidine in the G2 formed a thicker hybrid layer, and in the G4 it acted to close the interface. It was concluded that the quality of the bonding interface of indirect ceramic restorations is related to the type of adhesive system used.

Introduction

The indirect ceramic restorations, over the past decades, have been improved in order to better their physical and aesthetic properties. The evolution of these ceramic systems improved the ability to reproduce the characteristics of natural teeth and its mechanical strength. Therefore, the mechanical characteristics have enabled improvements in many clinical situations, increasing their indications. The longevity of this interface depends on several factors, including the surface treatment and luting agents that can contribute to the best closure of these edges and a seamless, dentin-ceramic system integration, thus, constituting the so-called monoblock.

Due to the expulsive characteristics of the preparations for indirect ceramic restorations, the adhesive systems become essential for the bonding between the restoration and the remaining tooth structure. But, the durability and effectiveness of the accession process formed by the cement-ceramic interface and the tooth-adhesive system have still been considered a concern for researchers.

In the process of indirect adhesive restoration, it is essential to use the acid etching in the interface formed between the dentin and the adhesive system. Its function is to activate a group of enzymes called metalloproteinase (MMPs), which contribute to the degradation of exposed collagen fibrils and, consequently, to the dentin-resin interface.

The Metalloproteinases are located within the mineralized dentin matrix, corresponding to a group of 23 enzymes capable of degrading collagen fibers exposed on the bonding interface. This degradation is due to the enzymatic activity of the metalloproteinases. The collagenolytic and gelatinolytic activities of the MMPs present in the dentin can be inhibited by chemical actions on the proteases, such as chlorhexidine digluconate increasing the longevity of the adhesion to the dentin. Chlorhexidine solutions have the ability to completely inhibit the activity of these enzymes even in small concentrations (0.03%) and can be applied to the dentin surfaces, remaining in contact for 30 to 60 seconds. In vitro and in vivo studies have shown good results in inhibiting the degradation of hybrid layers during the application of chlorhexidine digluconate (2%) on etched dentin with phosphoric acid and the prior use of a conventional single-component adhesive system.

The conditioning and demineralization of the tooth surface depend on the type, concentration, and time of the application of the acid used. The main mechanism of adhesion of current adhesive systems is based on the infiltration and subsequent polymerization of resin monomers in the surface region of the dentin, previously demineralized by acids, forming a substrate called the hybrid layer. The adhesive systems can be divided into two groups: conventional (two or three steps) and self-etching (one or two steps). The conventional adhesive systems use the total acid conditioning technique called total-etch that is to completely remove the smear layer, followed by the application of a primer or an adhesive associated with this. A major problem related to this technique is that...
the total depth of demineralized tissue is not always equally infiltrated by the monomers, providing for the existence of an unhybridized demineralized zone, and may cause nanoleakage and deterioration of the adhesive interface with postoperative hypersensitivity\(^9,10\). In self-etching adhesives, the technique used is self-etching; simpler and faster, it decreases clinician steps, having the advantage of eliminating the step of rinsing the conditioner and drying the substrate required by the conventional technique or total-etch, constituting, this way, a save of clinical time \(^3\). The self-etching adhesive system includes acidic primers that condition the dentine surface while the monomers infiltrate themselves to form a homogeneous hybrid layer, with a lower solution of continuity and minimizing the problem of nanoleakage \(^10\). However, few studies have investigated the benefits of chlorhexidine in slowing the deterioration of adhesive interfaces produced by these self-etching systems.

The objective of this study was to analyze the morphology of the dentin-adhesive system bonding interface by scanning it with an electron microscope while using the two adhesive systems, a total-etch and a self-etch, with and without the use of 2 % chlorhexidine.

**Methods**

For the experiments, four human third molars, recently extracted from the Surgery Clinic of UniFOA, were used with the approval of CoEPS CAAE-0061.0.446.000-10. The molars had their roots sectioned 2 mm below the cementoenamel junction. Then, using a blade with the aid of a metallographic cutter (ISOMET 1000, Buehler Ltd., Lake Bluf, IL, USA), equipped with a diamond disc (n.11-4254, Buehler Ltd., Lake Bluf, IL, USA), under constant lubrication (300 rpm and 200gf), the surface of the occlusal enamel of the teeth was removed, leaving the whole occlusal portion of dentin. The surfaces were inspected with a stereomicroscope (Model SZX7, Olympus, São Paulo, Brazil) with a 30-fold increase to confirm the absence of any remaining glaze. Next, the dentin surface was abraded in an automatic polisher (Ecomet 6/Automet, Buehler) using a sequence of carbide sandpaper of silicon granulation # 180, #240, and # 400 with copious irrigation until the exposure of an area of ??dentin approximately 4 mm in diameter (Fig. 1).

In this dentin surface, two grooves were made, one in the mesiodistal direction and another in the buccolingual direction, dividing this surface into four areas: mesiobuccal (MB), distobuccal (DB), mesiolingual (ML), and distolingual (DL).

Two different adhesive systems were used: the monolithic conventional Single Bond Adper 2 (3M ESPE, St. Paul, MN, USA) and the one-bottle self-etching adhesive system Adper Easy One (3M), along with 35% phosphoric Acid (Scotchbond Etchant, 3M ESPE, St. Paul, MN, USA), 2% Chlorhexidine (3M), and resin cement RelyX ARC (3M).

The samples were divided into four groups with different surface treatments:

**G1** - MV area of the samples - the dentin was conditioned for 15 seconds with 35% phosphoric acid, washed for 15 seconds, and dried with absorbent paper towels to obtain a wet surface. Two consecutive layers of the adhesive Single Bond Adper 2 were applied, each being subjected to air jets to remove the solvent, photopolymerized, and applied a layer approximately 2mm thick of resin cement RelyX ARC, and later photopolymerized for 40 seconds.

**G2** - DV area of the samples - the dentin was conditioned for 15 seconds with 35% phosphoric acid, washed for 15 seconds, and dried with absorbent paper towels to obtain a wet surface. Then, the conditioned dentin surface was moistened with 2% chlorhexidine digluconate for 30 seconds and dried with absorbent paper towels. Next, two consecutive layers of the adhesive Single Bond Adper 2 were applied, each being subjected to air jets to remove the solvent, photopolymerized, and applied a layer approximately 2mm thick of resin cement RelyX ARC, and later photopolymerized for 40 seconds.

**G3** - ML area of the samples - An active application of the Adper Easy One adhesive was carried out on the dry dentin surface for 20 seconds, with the aid of a disposable applicator. Then, air jets were used for 5 seconds on the dentin surface, photopolymerized for 10 seconds, and applied a layer approximately 2mm thick of resin cement RelyX ARC, and later photopolymerized for 40 seconds.

**G4** - DL area of the samples - The dentin was moistened with a solution of 2% chlorhexidine digluconate for 30 seconds and dried with absorbent paper towels. Next, an active application of the Adper Easy One adhesive system was carried out on the dentin surface for 20 seconds, with the aid of a disposable applicator. After that, the dentin was dried with air jets for 5 seconds, photopolymerized for 10 seconds, and applied a layer approximately 2mm thick of resin cement RelyX ARC, and later photopolymerized for 40 seconds.

While an area received the surface treatment, the others were protected with thread sealing tape. Later,
using a blade with the aid of a metallographic cutter (ISOMET 1000, Buehler Ltd., Lake Bluf, IL, USA), equipped with a diamond disc (n.11-4254, Buehler Ltd., Lake Bluf, IL, USA), under constant lubrication (300 rpm and 200gf), two mesiodistal cuts were made, parallel to the mesiodistal groove already made: the first cut 2mm from the mesiodistal buccal groove and the second cut 2mm from the second mesiodistal lingual groove; thus, it exposed the bonding interface formed by the dentin and by the adhesive systems used (Fig. 2).

For the evaluation at SEM, these bonding interfaces were prepared and cleaned following a protocol of manual polishing with carbide sandpaper of silicon granulation 4.000. They were metallographically polished with self-adhesive cloths and alumina (0.5 and 1µm) and demineralized with 50% phosphoric acid for 3 seconds. After each of these steps, the samples were packed in eppendorfs with distilled water and placed in an ultrasound. After that step, the samples were prepared for SEM (EVO MA10 of Carl Zeiss) through dehydration in an ascending series of alcohol (70%, 80%, 90%, and absolute) for 15 minutes in each solution, except in the absolute alcohol where they remained for 30 minutes. Then, they were fixed in stubs, coated with gold, and taken to the reading at SEM. The four areas of each sample (MB-DB-ML-DL) were evaluated, always starting at the center of each.

Results and Discussion

The micrographs of the groups 1, 2, 3, and 4 show the constituent biological structures of tooth structure: resin cement (RC), adhesive system (SA), hybrid layer (CH), dentin (D), dentin interface-adhesive system. They were analyzed morphologically.

During an adhesive cementation, several bonding interfaces are formed; but, if they occur between the bottom of hybrid layer and the underlying dentin, the clinical consequences are inevitable, since the dentin will not become sealed enough risking bacterial invasion, dentinal sensitivity, and irritation to the pulp tissue. These failures usually indicate that there was no infiltration of the monomers, and that they did not sustain the collagen fibers (6,11). This paper uses two types of adhesive systems with different protocols to apply the acid etching. The conventional adhesive system, previously having used the acid etching, completely removes the smear layer, and the whole area that was demineralized will not necessarily be infiltrated by resin monomers (12,13). The self-etching adhesive system used, also known as all in one, was a single step. This adhesive combines acid, primer, and adhesive in a single application; this characteristic of simplifying clinical steps determines a greater commitment of its effectiveness (12, 13). Along with the adhesive systems, new adhesion techniques are proposed in the pursuit for the quality and durability of these bonding interfaces. Among these techniques, the use of the chlorhexidine digluconate solution, after acid etching, acts as an inhibitor of protein present in the saliva and dentin (5, 13, 14). The protocol for use of the chlorhexidine and the removal the excess of this solution must also be evaluated. In this study, its application was after the use of phosphoric acid in the conventional adhesive system technique and before the application of the self-etching adhesive system, a fact that should be taken into consideration because the remaining amount of chlorhexidine on the dentin may positively or negative influence the surface moisture in the substrate (15).

In this work, the micrographs suggested that both of the adhesive systems were capable of forming a hybrid layer, however, with different thicknesses, confirming the work given in the references (5, 14, 16, 17).

The groups 1 and 2 used the conventional adhesive system with and without the chlorhexidine digluconate. When the conventional adhesive system was used with chlorhexidine (G2), it showed a thick and thin hybrid layer, and the bonding interface dentin-adhesive system was regular and closed with definite resin tags (Fig. 3 and 9) (18, 19). However, the G1 group showed less thickness and irregularity in the hybrid layer, with resin tags without definition (Fig. 4 and 10). The results of the G1 group may be due to the difficulty of this type of adhesive system to evaporate the water present in the dentin, affecting the conversion of monomers into polymers, thereby, quickly degrading the adhesive interface (12, 13). Nevertheless, in the G2 group, the use of chlorhexidine might have contributed to improve the infiltration of the monomers since this substance inhibits the MMPs present in the saliva and dentin, improving the stability of the collagen fibers and, consequently, forming a more even hybrid layer (13, 20, 21).

The groups 3 and 4 used the self-etching adhesive system also with and without the chlorhexidine. This group showed that the smear layer was embedded in the adhesive interface, forming a slightly thick hybrid layer (Fig. 5, 6, 7, 8) (13, 17, 12, 22, 23). However, in the G3 group, the resin tags were not evident and had open interfaces (Fig. 6 and 7) which may be due to the acidity concentration and water present in the solution of self-etching adhesives, which can result in
increased hydrophilicity in regions of incomplete polymerization and subsequent degradation of the interfaces (11, 13). In the group associated with the self-etching adhesive and chlorhexidine (G4), the closure of the interfaces with well defined resin tags was observed in most of the samples (Fig. 5 and 8). Maybe the closure of the bonding interfaces in the G4 group is due to the greater preservation of the collagen fibers by the chlorhexidine solution (15).

Summary

The two adhesive systems used were capable of forming hybrid layers with different thicknesses. In the groups that used chlorhexidine, definite resin tags were observed. The association of the chlorhexidine with the selfETCH adhesive system improved the integrity of this bonding interface. The quality of the bonding interface is directly related to the type of adhesive system used.

References


Illustrations

Illustration 1

Fig 1- Exposure of an area of dentin approximately 4 mm in diameter
Illustration 2

Fig. 2- Disclosure of 1st cut and 2nd cut
Illustration 3

Fig.3- Group 2- SB + CCL
Illustration 4

Fig.4- Group 1 - SB + SCL
Illustration 5

Fig.5 - Group 4 - EO + CCL
Illustration 6

Fig.6- Group 3- EO + SCL
Illustration 7

Fig. 7 - Group 3- EO + SCL
Illustration 8

Fig.8- Group 4- EO + CCL
Illustration 9

Fig.9- Group 2- SB + CCL
Illustration 10

Fig. 10- Group 1- SB + SCL