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Mestrado Integrado em Medicina Dentária

Effect of dentin surface treatment with AquaCare system prior to bonding

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TABLE OF CONTENTS

Abstract ... 3
Resumo ... 4
Introduction ... 5
Materials and Methods ... 7
1. Preparation of Specimens ... 7
2. Dentin Pretreatment ... 7
3. Scanning Electron Microscopy (SEM) ... 9
Results ... 10
Discussion ... 17
1. Dentin Adhesion ... 17
2. Air abrasion with aluminum oxide ... 18
3. Air abrasion with bioactive glass ... 20
4. Considerations and limitations ... 21
Conclusion ... 22
Acknowledgments ... 23
References ... 24

Abstract

Introduction: Several techniques are described to prepare dentin for adhesion. Air abrasion is a mechanical pretreatment used to introduce surface modifications and has been suggested to clean dentin surface prior to bonding procedures. This study aimed to assess the effects of air abrasion with two AquaCare powders (Al_2O_3 and *Sylc*) on dentin, as well as on the interface to a self-etch adhesive (Clearfil™ SE Bond II - CSE) through morphological characterization using scanning electron microscopy (SEM).

Materials and Methods: Two extracted human molars were selected and cross-sectioned to obtain two dentin discs, then fractured into four fragments each. According to dentin surface treatment, the samples (1/4 disc) were divided into eight experimental groups: Control-1, PC, PC-Primer, PC-Bond, Control-2, *Sylc*, *Sylc*-Primer and *Sylc*-Bond. After air abrasion and adhesive procedures, specimens were prepared, coated and observed through SEM.

Results: In control groups, the presence of smear layer, occluding most dentin tubules, is evident. However, after air abrasion with 29 µm Al_2O_3, the presence of an irregular, amorphous, dense and compact heavily packed smear-layer with most tubules obstructed can be identified. In PC-Primer group, a certain amount of debris inside the tubules and crack-like alterations produced by the abrasive can be noticed. In PC-Bond group, the adhesive interface is well-defined and small resin tags are visible.

SEM images of *Sylc* group reveal a dense, compact and amorphous smear layer responsible for the occlusion of dentin tubules. In *Sylc*-Primer group, the CSE primer appears to have dissolved and integrated the smear layer, thus opening dentin tubules, which are fully exposed and visible. In *Sylc*-Bond group, a smooth CSE surface is observed alongside the presence of some crater-like *loci*.

Conclusion: Dentinal surface treatment with AquaCare powders influences the smear layer and the adhesive interface obtained with the CSE adhesive system.

Keywords: Air abrasion; Aluminum oxide; Bioactive glass; Dentin; Dentin adhesion; Scanning electron microscopy.

Resumo

Introdução: Na literatura estão descritas várias técnicas para preparação da superfície dentinária previamente aos procedimentos adesivos. O jateamento é um tratamento mecânico usado para introduzir modificações na superfície e tem sido sugerido como um método de limpeza da superfície dentinária antes da realização de procedimentos adesivos. O objetivo deste trabalho é avaliar os efeitos do jateamento com dois pós do sistema AquaCare (Al_2O_3 e *Sylc*) na dentina, bem como na interface de um adesivo auto-condicionante (Clearfil™ SE Bond II - CSE) através da caracterização morfológica com recurso a microscopia eletrónica de varrimento (MEV).

Materiais e Métodos: Dois molares humanos foram seccionados de forma a obter dois discos de dentina, posteriormente fraturados em 4 fragmentos. As amostras (1/4 disco) foram divididas em 8 grupos experimentais de acordo com o tratamento da superfície dentinária: Control-1, PC, PC-Primer, PC-Bond, Control-2, *Sylc*, *Sylc*-Primer and *Sylc*-Bond. Após a realização do jateamento e dos procedimentos adesivos, as amostras foram preparadas para observação.

Resultados: Nos grupos controlo, é evidente a presença de *smear layer* a obliterar a entrada da maioria dos túbulos dentinários. Após jateamento com 29 µm Al_2O_3 é possível identificar uma *smear layer* irregular, amorfa, densa e fortemente compactada, com a maioria dos túbulos obstruídos. No grupo PC-Primer é possível observar a presença de alguns detritos no interior dos túbulos e alterações semelhantes a crateras produzidas pelo agente abrasivo. No grupo PC-Bond a interface adesiva está bem definida e pequenos *resin tags* podem ser identificados.

Nas imagens de MEV do grupo *Sylc* pode observar-se uma *smear layer* densa, compacta e amorfa responsável pela oclusão dos túbulos dentinários. No grupo *Sylc*-Primer, parece ter ocorrido dissolução e integração da *smear layer* com abertura e exposição completa dos túbulos dentinários. No grupo *Sylc*-Bond uma superfície lisa criada pelo CSE está presente e alguns locais semelhantes a crateras podem ser identificados.

Conclusão: O tratamento da superfície dentinária com o sistema AquaCare influencia não só o tipo de *smear layer*, mas também a interface adesiva obtida com o sistema adesivo CSE.

Palavras-chave: Adesão dentinária; Dentina; Jateamento; Microscopia eletrónica de varrimento; Óxido de alumínio; Vidro bioativo.

Introduction

The longevity of a bonded restoration is closely linked to the adhesive system's effectiveness.[1,2] Notwithstanding, achieving a high-quality, stable hybrid layer remains the main challenge in dentinal adhesion, as dentin bonding proves to be more complex due to a higher amount of organic content and presence of smear layer. Moreover, the hydrophilicity of this substrate interferes with its interaction with adhesive systems.[3]

The key bonding mechanism consists of an exchange process involving the replacement of inorganic tooth substrate by resin monomers that, upon polymerization, become micromechanically interlocked in the previously generated porosities. On dentin, this process is called 'hybridization' and involves the formation of the hybrid layer.[3,4] The potential benefit of an additional chemical interaction between specific monomers and calcium in hydroxyapatite is also described as an essential mechanism in dentinal adhesion.[5,6]

There are two main adhesive strategies used to create effective dentin bonding: etch-and-rinse and self-etch adhesives.[6]

The etch-and-rinse strategy involves the prior application of phosphoric acid, which promotes demineralization of dentin and completely removes the smear layer, leading to exposure of the collagen fibrils.[3,7] On the other hand, self-etching adhesives do not require a separate etching step. They contain non-rinse acidic monomers that dissolve the smear layer and simultaneously condition and prime the tooth surface to the same depth, theoretically ensuring complete penetration of the monomers in the exposed collagen.[3,4,6,8]

The latest generation of adhesives is known as "universal" or "multi-mode" and can be applied according to different adhesion strategies: etch-and-rinse, self-etch, or selective enamel-etch. This versatility allows the clinician to decide which approach suits better each clinical situation.[1,9]

Smear layer greatly varies in quantity and quality depending on dentin mechanical and chemical pretreatments, which may jeopardize the clinical bonding effectiveness to dental substrates. Thus, dentin surface treatments for smear layer cleaning, such as its complete removal, dissolution, replacement or modification, are pivotal steps prior to restorative bonding procedures.[4,10,11]

Within the same scope, air abrasion techniques, which comprise abrasive particles in a fine stream of compressed air, have been suggested as a dentin surface cleansing method. As the particles collide with dentin, their kinetic energy is released, resulting in the fracture of microscopic dentin fragments caused by ablation of small amounts of tooth structure.

Consequently, air abrasion increases surface roughness and, thus, the available area for adhesion.[2,10,12,13]

The choice of powders used to perform this type of dentin pretreatment may influence the quality and durability of the tooth-restoration interface.[14] Among the abrasive agents, aluminum oxide (Al_2O_3) is the most commonly used in air abrasion units.[15] Notwithstanding, a material that may promote an inhibitory effect on endogenous collagenolytic/gelatinolytic activity would prove effective as means of eschewing bond degradation that occurs over time.[16] Air abrasion with bioglass 45S5 (BAG), a bioactive calcium/sodium phosphate-phyllosilicate glass, has been used as an alternative to Al_2O_3 once it appears to have the ability to create a bioactive smear-layer which may react with body fluids, encouraging the formation of hydroxyapatite and, therefore, favoring the remineralization of dental hard tissues.[16–18]

AquaCare (AquaCare, Velopex International, London, UK) is a dental air abrasion unit that uses different powders for tooth surface preparation, among which a 29 μm aluminum oxide (Al_2O_3) and a bioactive glass powder (*Sylc*) are the most widespread. There is some speculation among clinicians about the contribution of these powders to improving bond strength, but the scarcity of studies on the effects of these surface treatments renders the technique controversial and with little scientific foundation for clinical application.

Thus, this study aimed to assess the effects of air abrasion with two AquaCare powders (Al_2O_3 and *Sylc*) on dentin, as well as on the interface to a self-etch adhesive (Clearfil™ SE Bond II - CSE) through morphological characterization using scanning electron microscopy (SEM).

The alternative hypothesis of this study is that dentin pretreatment with AquaCare powders has significant effects on dentin morphology.

Materials and Methods

1. Preparation of Specimens

This study was approved by the Ethics Committee of the Faculty of Medicine - University of Coimbra (notification CE001/2013).

Two recently extracted intact human molars were selected for this study and immediately immersed in distilled water for one week. Only teeth clinically free of caries and restorations were included. Any residual soft tissue and debris were removed from the roots with a curette. The teeth were then stored in an aqueous solution of 0.5% chloramine at 8ºC for six weeks before the experimental procedure. A silicone putty mold (Hydrorise, Zhermack, Rovigo, Italy, Lot: 305675, Exp: 2021/11) with a cylindrical shape was used for the partial inclusion of the roots in autopolymerizing acrylic resin blocks (Autopolimerizable Acrylic Resin, Schmidt Laboratory, Madrid, Spain, Lot: 47975, Exp: 2021/11).

The occlusal enamel was removed and two coronal dentin discs with 0.8 mm thickness were obtained from a perpendicular cut to the long axis of the teeth with a low-speed (300 rpm at 0.050 mm/s) diamond saw in a precision cutting machine (Accutom-5, Struers, Ballerup, Denmark), under continuous water cooling.

Dentin surfaces were subsequently manually prepared using silicon carbide abrasive paper in an ascending grits series (120, 240, 600 grits, according to ISO/DTS 11405), under constant irrigation and circular motion, to produce and standardize the smear layer. Then, the samples were rinsed with water.

After accomplishing these procedures, each dentin disk was fractured into four fragments.

2. Dentin Pretreatment

Dentin specimens were divided into eight experimental groups, according to dentin surface treatment:

Control-1: dentin with smear layer

PC: dentin with smear layer abraded with AquaCare 29 μm Al_2O_3

PC-Primer: dentin with smear layer abraded with AquaCare 29 μm Al_2O_3 + primer CSE

PC-Bond: dentin with smear layer abraded with AquaCare 29 μm Al_2O_3 + adhesive resin CSE

Control-2: dentin with smear layer

***Sylc*:** dentin with smear layer abraded with AquaCare BAG

***Sylc*-Primer:** dentin with smear layer abraded with AquaCare BAG + primer CSE

***Sylc*-Bond:** dentin with smear layer abraded with AquaCare BAG + adhesive resin CSE

The distribution of the study groups is represented in Figure 1.

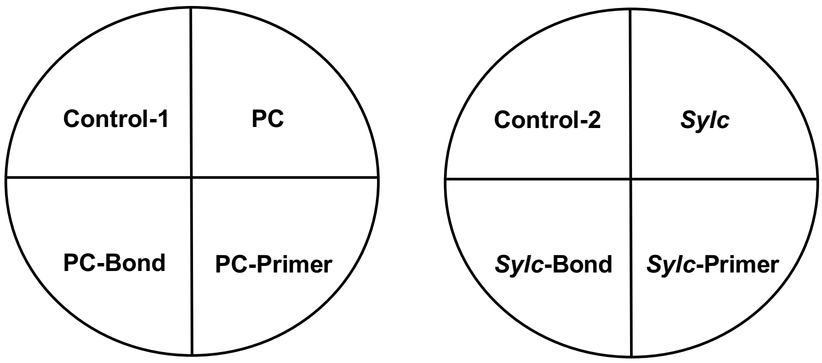

Figure 1: Distribution of the study groups

The AquaCare air abrasion unit (AquaCare, Velopex International, London, UK) was used with an air pressure of 4 bar for 10 seconds, under continuous irrigation with AquaSol (AquaSol, Velopex International, London, UK, Lot: 10049, Exp: 4/2022). The nozzle was positioned at a distance of 1 cm from the dentin surface with an angle of approximately 45º. Specimens were then thoroughly rinsed with water for 30 seconds and air-dried.

The adhesive system Clearfil™ SE Bond II was applied according to the manufacturers' instructions, following each experimental group-specific methodology. The primer was actively applied using a microbrush for 20 seconds; then the surface was gently dried with mild air to evaporate the solvent in groups PC-Primer, *Sylc*-Primer, PC-Bond and *Sylc*-Bond. The adhesive resin was then actively applied for 20 seconds and gently dried to produce a uniform bonding film in PC-Bond and *Sylc*-Bond groups. The adhesive system was light-cured for 20 seconds with a light-curing unit (Bluephase® Style 20i, Ivoclar Vivadent, Schaan, Liechtenstein, 1200 mW/cm^2). The composition, lot number and expiration date of the adhesive system and AquaCare powders used in this study are described in Table 1.

Groups in which bonding resin was used were coated with a glycerin gel (Liquid Strip, Ivoclar Vivadent, Schaan, Liechtenstein) and light-cured for 10 seconds (Bluephase® Style 20i, Ivoclar Vivadent, Schaan, Liechtenstein, 1200 mW/cm^2) to remove the oxygen inhibited layer.

Table 1: Composition, lot number and expiration date of Clearfil™ SE Bond II and AquaCare powders

Material	Composition	Lot number	Expiration date
Clearfil™ SE Bond II 2-step self-etch adhesive (Kuraray Noritake Dental Inc., Okayama, Japan)	**Primer:** 10-Methacryloyloxydecyl dihydrogen phosphate (MDP), 2-Hydroxyethyl methacrylate (HEMA), Hydrophilic aliphatic dimethacrylate, dl-Camphorquinone, water **Bond:** MDP, Bisphenol A diglycidylmethacrylate (Bis-GMA), HEMA, Hydrophobic aliphatic dimethacrylate, dl-Camphorquinone, Initiators, Accelerators, Silanated colloidal silica	000119	07-2023
pro*Cut* 29 µm Al_2O_3 (Medivance Instruments Limited, London, UK)	Aluminum oxide	200618	-
pro*sylc* *Sylc* bioglass 45S5 (Dentofex Research Ltd., London, UK)	45S5 Bioglass: Silicon, calcium, sodium, phosphorus and oxygen	021018	-

3. Scanning Electron Microscopy (SEM)

For SEM analysis, the specimens from groups Control-1, PC, PC-Primer, Control-2, *Sylc* and *Sylc*-Primer were firstly fixed in 2.5% glutaraldehyde in 0.1M phosphate-buffered saline (PBS) (0.1M, pH 7.4) for 48 hours at 4ºC and then immersed in three baths of 0.1M PBS for 30 minutes each. The fixed specimens were posteriorly dehydrated in ascending ethanol concentrations (30%, 50%, 70%, 80%, 95%, 100% and 100%) for 20 minutes per step, followed by immersion in 0.5 mL hexamethyldisilazane (HMDS) until its complete evaporation. All specimens were stored in absorbent paper with silica for two days at room temperature.

Each dried dentin sample was fractured into halves to evaluate the specimens in a longitudinal and transversal cut. Subsequently, each fragment was mounted on aluminum stubs using carbon glue and sputter-coated with gold-palladium (Polaron E5000 Sputter-Coater, Polaron Equipment Limited, Watford, UK) for SEM (SEM, Hitachi S-4100, Tokyo, Japan) observation mostly under 2000x magnification and in some specific situations under 5000x and 10000x.

Results

SEM observations

1. **Control-1 group**

Representative micrographs of Control-1 group, corresponding to dentin with smear layer, can be observed in Figure 2 in a longitudinal view, whereas Figures 3 and 4 present a transversal view.

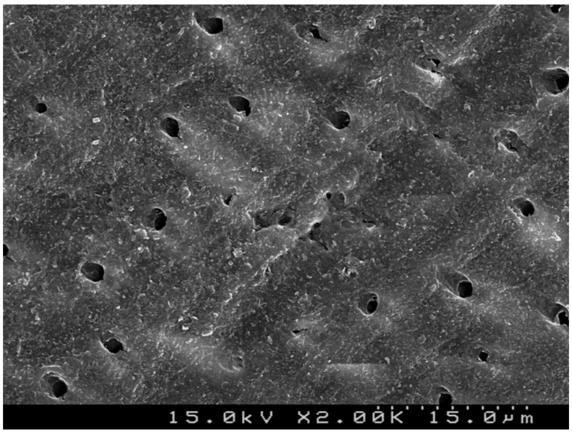

Figure 2: SEM image of Control-1 group, longitudinal view (2000x)

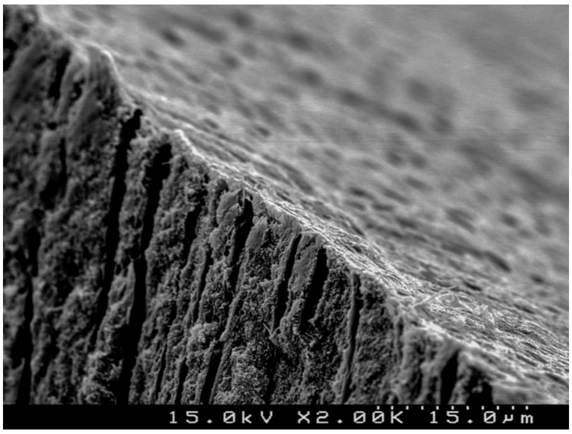

Figure 3: SEM image of Control-1 group, transversal view (2000x)

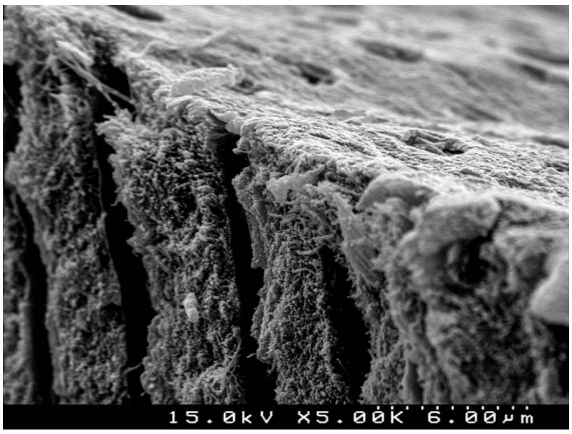

Figure 4: SEM image of Control-1 group, transversal view (5000x)

In a longitudinal view, the presence of intertubular smear layer, occluding most dentin tubules, is evident. Some tubules are visibly open and do not present smear layer or smear plugs. In a transversal view, it is possible to observe a high density of tubules occluded by smear layer and smear plugs, as well as tubular, peritubular and intertubular dentin.

2. PC group

PC group, corresponding to dentin air abraded with AquaCare 29 µm Al_2O_3 can be observed in Figure 5 in a longitudinal view, whereas Figure 6 presents a transversal view.

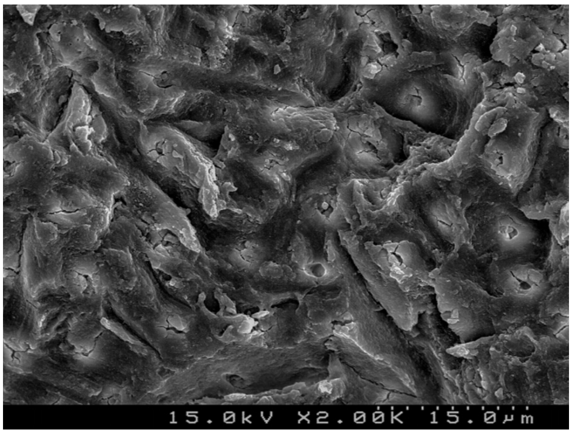

Figure 5: SEM image of PC group, longitudinal view (2000x)

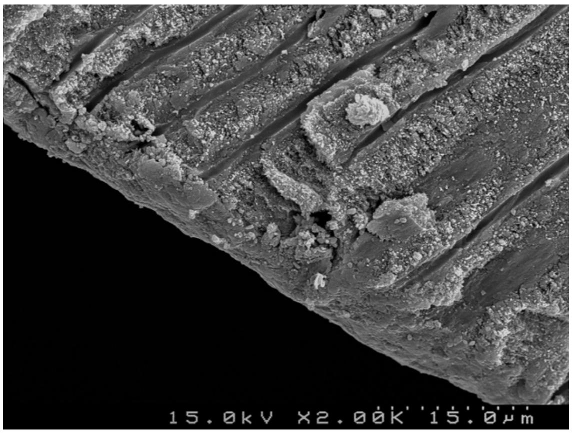

Figure 6: SEM image of PC group, transversal view (2000x)

In SEM images of this group, the presence of an irregular, amorphous, dense and heavily packed smear layer can be identified in both longitudinal and transversal views. The longitudinal view displays a rough, heavily cratered surface, with most tubules obstructed.

3. PC-Primer group

The representative micrographs of PC-Primer group, corresponding to dentin air abraded with AquaCare 29 µm Al_2O_3 and followed by the application of CSE primer, are represented in Figures 7 and 8 in a longitudinal perspective, while Figure 9 was taken in a transversal view.

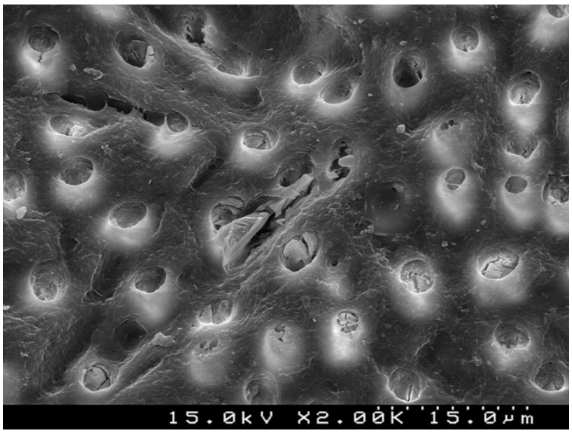

Figure 7: SEM image of PC-Primer group, longitudinal view (2000x)

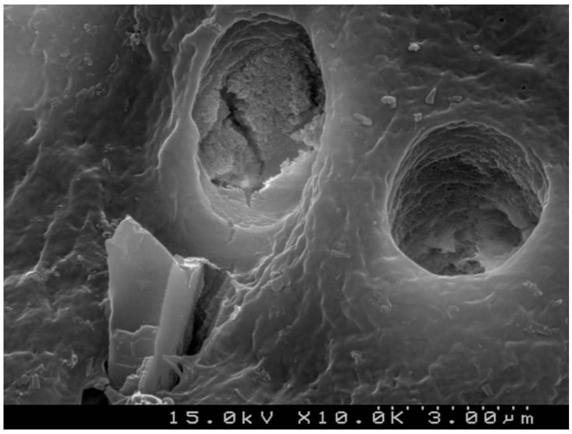

Figure 8: SEM image of PC-Primer group, longitudinal view (10000x)

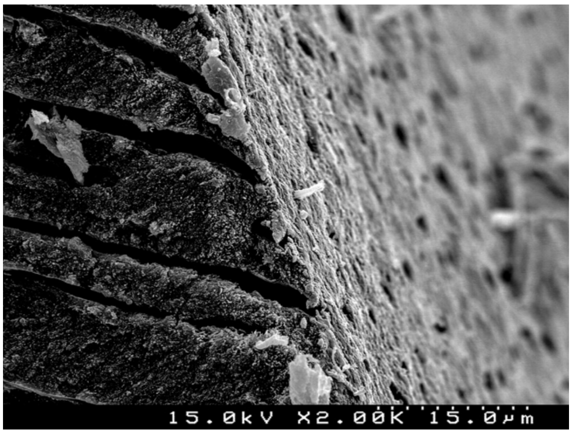

Figure 9: SEM image of PC-Primer group, transversal view (2000x)

In all longitudinal and transversal sections, open dentin tubules are visible, while some present smear-plugs. Moreover, a certain amount of debris inside the tubules and crack-like alterations produced by the abrasive can be identified. In Figures 7 and 8, two debris particles are seen piercing through intertubular dentin and lodging between dentinal tubules.

4. **PC-Bond group**

Figures 10 and 11 represent the micrographs of PC-Bond group corresponding to AquaCare 29 µm Al_2O_3 air abraded dentin followed by CSE application.

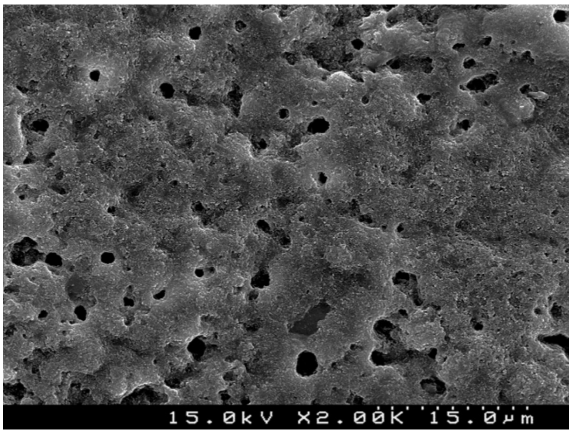

Figure 10: SEM image of PC-Bond group, longitudinal view (2000x)

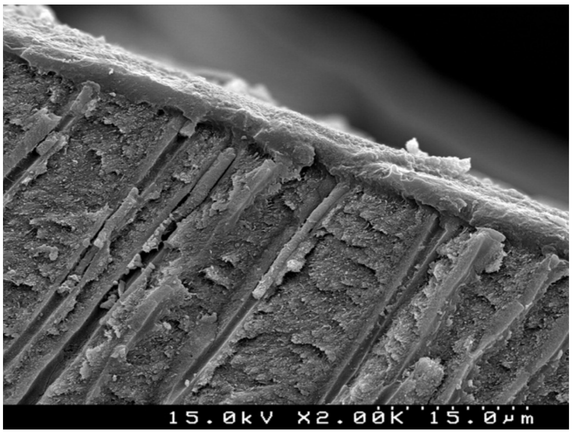

Figure 11: SEM image of PC-Bond group, transversal view (2000x)

In a transversal view of PC-Bond group, the adhesive interface is well-defined and small resin tags are visible. The longitudinal view exhibits a standard adhesive layer with some spots of discontinuity.

5. **Control-2 group**

Control-2 group, corresponding to dentin with smear layer, is represented in Figure 12 in a longitudinal view. As in Control-1 group, the presence of smear layer can be noticed.

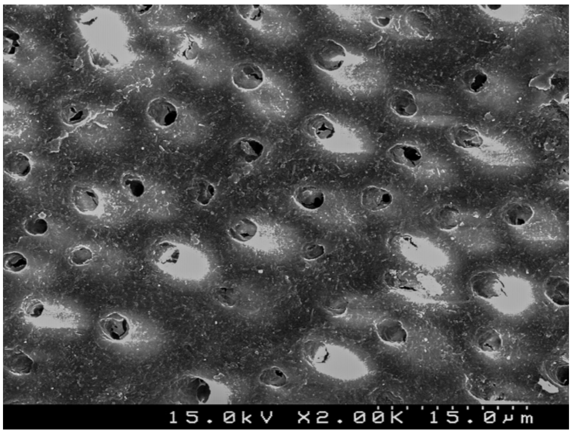

Figure 12: SEM image of Control-2, longitudinal view (2000x)

6. ***Sylc* group**

Sylc group is represented in Figures 13 and 14 in a longitudinal perspective, while Figure 15 was taken in a transversal view.

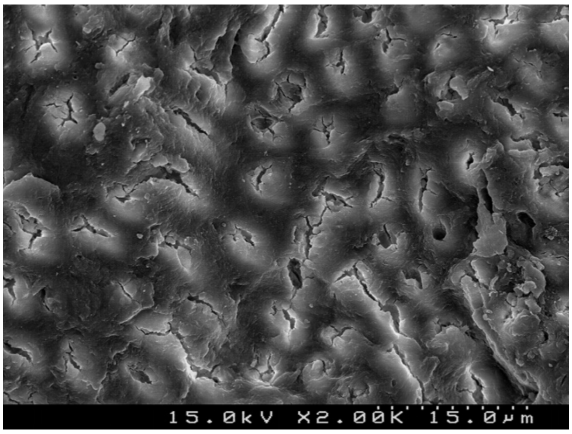

Figure 13: SEM image of *Sylc* group, longitudinal view (2000x)

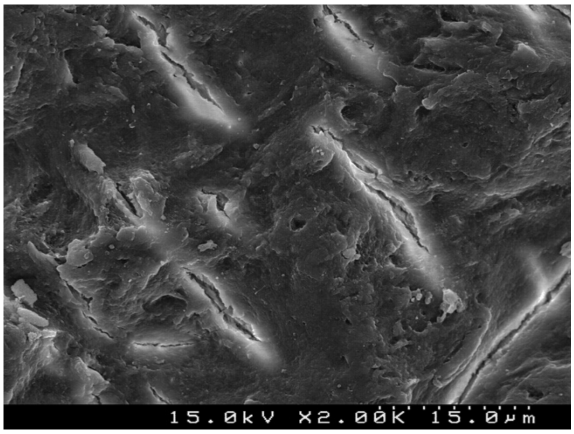

Figure 14: SEM image of *Sylc* group, longitudinal view (2000x)

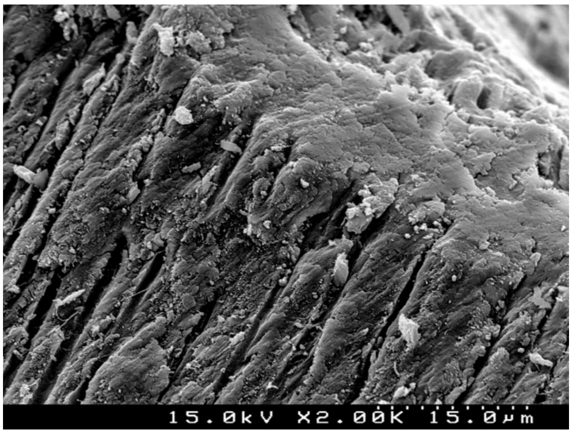

Figure 15: SEM image of *Sylc* group, transversal view (2000x)

The SEM images of *Sylc* group reveal, in a longitudinal view, a dense, compact and amorphous smear layer responsible for the occlusion of dentin tubules. A pattern of micro-fissures is widespread through the longitudinal sample. An exceptionally compact smear layer is visible in a transversal view, maintaining the morphological characteristics described for the longitudinal observation.

7. Sylc-Primer group

Figures 16, 17, 18 and 19 represent the micrographs of *Sylc*-Primer group, corresponding to *Sylc* air abraded dentin followed by the application of CSE primer.

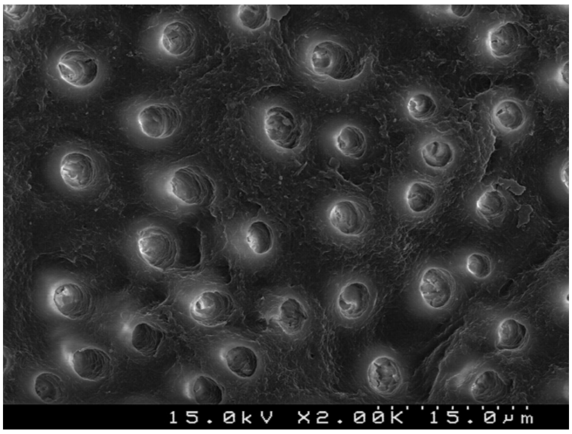

Figure 16: SEM image of *Sylc*-Primer group, longitudinal view (2000x)

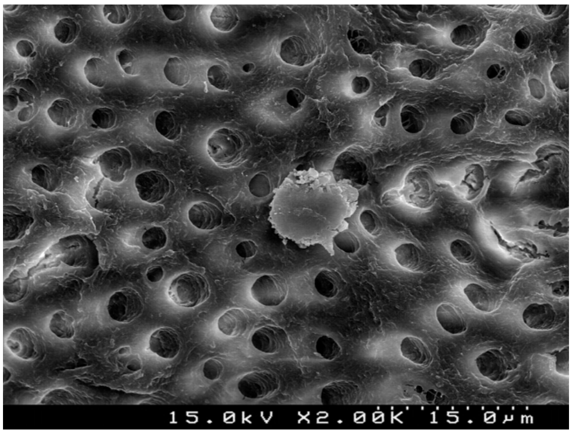

Figure 17: SEM image of *Sylc*-Primer group, longitudinal view (2000x)

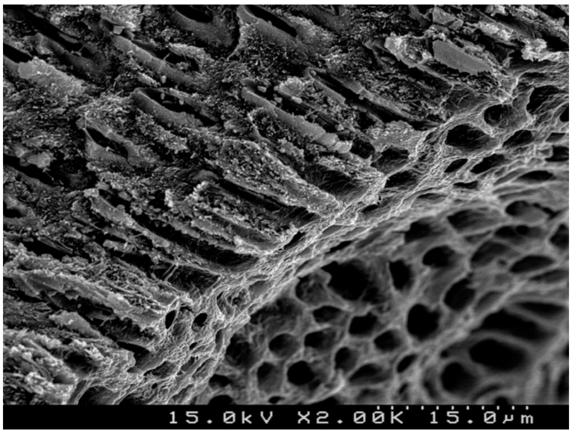

Figure 18: SEM image of *Sylc*-Primer group, transversal view (2000x)

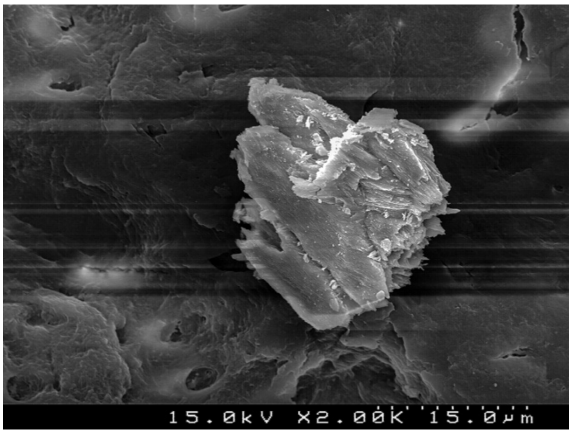

Figura 19: SEM image of a *Sylc* particle (2000x)

In both longitudinal and transversal views of *Sylc*-Primer group micrographs, the CSE primer appears to have dissolved and integrated the smear layer, thus opening the dentin tubules, which are fully exposed and visible. As represented in Figures 17 and 19, detached particles with an irregular shape are present throughout the sample.

8. *Sylc*-Bond group

Sylc-Bond group, corresponding to *Sylc* air abraded dentin followed by CSE application, is represented in Figure 20 in a longitudinal perspective, whilst in Figure 21, a transversal cut can be observed.

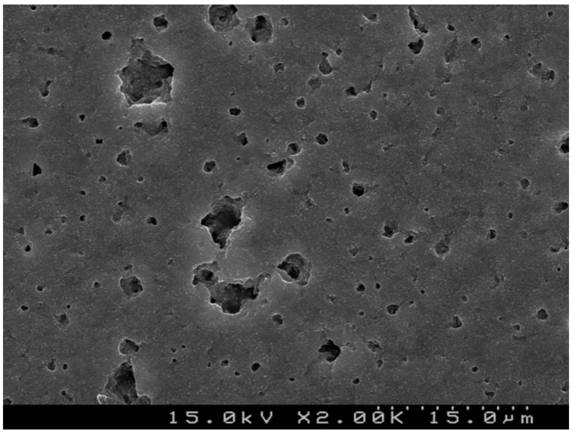

Figure 20: SEM image of *Sylc*-Bond group, longitudinal view (2000x)

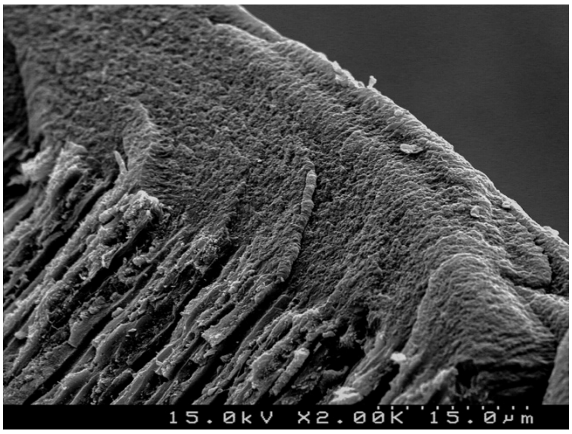

Figure 21: SEM image of *Sylc*-Bond, transversal view (2000x)

In the transversal view of *Sylc*-Bond group, the adhesive interface can be easily identified. Regarding the longitudinal view, a smooth CSE surface is observed alongside the presence of some crater-like *loci*.

Discussion

1. Dentin Adhesion

Adhesion durability between the adhesive system and teeth structures is critical to the longevity of bonded restorations.[2] Effectively bonding to a highly inorganic structure as enamel is a predictable procedure. Conversely, the creation of a reliable adhesion to dentin remains a challenging task due to the specific features of this tissue: presence of water, smear layer and high organic content.[10] Furthermore, dentin is intimately intertwined with pulpal tissue by means of numerous fluid-filled tubules, which, under constant outward pressure, render the exposed dentin surface naturally moist with intrinsic hydrophilicity that subsidizes the complexity of this substrate.[3]

Following cavity preparation, dentin is covered with an iatrogenically produced smear layer that creates a physical barrier obliterating the dentinal tubules' entrance and reducing their permeability to the penetration of adhesive systems. This debris layer must be dissolved or made permeable to improve monomer infiltration.[13] Depending on the preparation technique, smear layer may present different composition, thickness, morphology and degree of attachment to the underlying tooth structure.[3,4]

All dentin samples were manually prepared in the present study using silicon carbide abrasive paper of distinct grit sizes to obtain a uniform and standard smear layer. This technique provides a flat surface with fewer irregularities when compared with conventional cutting instruments and the standardized smear layer produced can be used with different surface treatments.[10] However, this method produces a looser smear layer and the presence of open dentin tubules is more likely to occur than if a bur provided it.[19]

In SEM observations of control groups, the presence of smear layer occluded the entrance of most dentin tubules. Some open tubules are spotted, suggesting that partial removal of the smear layer might have occurred. An analysis of dentin in the same disk used for other experimental groups (e.g., *Sylc*-Primer) also leads to the deduction that samples used present a very high number of tubules, corresponding to deep dentin, thus even though only a small percentage is visible, a significant overall number of tubules may be spotted despite most being covered by smear layer. In a study of Anja *et al.*, where the smear layer was also standardized with silicon carbide abrasive paper of different grit sizes, SEM images exhibit an intact smear layer with a small number of exposed dentin tubules.[10] The differences in the specimen fixation protocol may explain the distinct results obtained in these studies. The findings by Anja *et al*. are in accordance with Malkoc *et al.,* who found that untreated dentin surfaces were covered by an intact smear layer that occluded most dentin tubules.[5]

Etch-and-rinse adhesives are characterized by an initial etching step, followed by a compulsory rinsing procedure that completely removes the smear layer and smear plugs. This approach promotes dentin demineralization exposing a mesh of collagen that is nearly totally devoided of hydroxyapatite.(3,6) Nonetheless, clinical manipulation of phosphoric acid increases the technique sensitivity due to the rinsing-drying steps that can lead to a collapse of the collagen network.(1,3) Contrariwise, in self-etch adhesives, this sensitivity is significantly reduced as the primer dissolves the smear layer, demineralizing and infiltrating dentin simultaneously and to the same depth, thus decreasing the risk of collagen collapse and degradation.(4,20) Moreover, morphological features of the hybrid layer produced by self-etch adhesives depend on their functional monomers' interaction with dentin. According to their acidity, self-etch adhesives can be classified as strong, intermediate and mild, which determines their effect on smear layer dissolution and demineralization capacity.(4)

Clearfil™ SE Bond, used in this study, is a two-step “mild” self-etch system that partially demineralizes dentin, leaving a substantial amount of hydroxyapatite around the collagen fibrils. This approach seems to provide superior performance when bonding to dentin due to the presence of functional monomers (10-methacryloyloxydecyl dihydrogenphosphate - 10-MDP) that ionically bond to calcium in hydroxyapatite.(3,4,6) This chemical interaction may result in bonds more resistant to hydrolytic degradation, which leads to a longer-lasting marginal seal.(8)

Adhesion is not only influenced by the penetration of adhesive resin into dentin tubules but also by mechanical interlocking, surface adhesion and collagen network. Hence, the availability, quantity and quality of intertubular dentin plays a key role in adhesion, with its pristine condition being nowadays one of the primary concerns when bonding to dentin.(21)

2. Air abrasion with aluminum oxide

Air abrasion, defined as a surface mechanical pretreatment, increases roughness and the area for adhesion, improving the contact between adhesive and substrate.(1,10,21,22)

According to Rafael *et al*., this method promotes the preservation of dentinal tubules' original diameters and, consequently, the amount of available intertubular dentin.(21)

The majority of papers on the topic studied bond strength between restorative materials and air abraded dentin, with often contradictory results. Nevertheless, the present study only assessed the effects of AquaCare powders in dentin microstructure through SEM observation of air abraded specimens before and after priming and bonding procedures.

Our results demonstrate that PC group, air abraded with 29 µm Al_2O_3, is covered by an irregular, dense and amorphous smear layer, different from that found in the control group.

Furthermore, only some dentin tubules are opened. In agreement with these results, França *et al.* described the creation of a similar smear layer after air abrasion with 50 µm Al_2O_3.[2] Other studies reported the formation of an amorphous and irregular layer of debris with completely obliterated tubules after sandblasting with Al_2O_3.[5,15,23]

The efficacy of air abrasion systems might be influenced by several parameters: tip design, air pressure, type and size of abrasive particles, application time, angle and distance from the dentin surface.[24,25]

Some authors demonstrated that Al_2O_3 air abrasion with particles of different sizes induces similar changes on dentin surfaces, although smaller particles promoted a more retentive pattern. PC group exhibited a heavily cratered dentinal surface, compatible with these findings.[15,26,27]

In SEM analysis of PC-Primer group, CSE's primer dissolved the smear layer and exposed the dentin tubules previously occluded with a dense smear layer observed in PC group. Some smear plugs persist, consistent with the widely reported CSE self-etching pattern characterized by a limited demineralization of dentin compared to total-etching techniques, without complete removal of smear plugs or opening of all tubules.[22] Furthermore, in this group, crack-like alterations produced by the abrasive can be identified. These cracks on dentin surface were previously described by Rafael *et al.* and suggested to impact hardness and structural integrity of dentin.[21]

Another important aspect to consider is that AquaCare air abrasion unit was used in this study with the proprietary irrigation solution to shroud the abrasive particles. Notwithstanding, SEM images of PC-Primer group show some abrasive particles embedded in dentin, with some piercing through intertubular dentin and lodging in between dentinal tubules. It was previously reported that water shrouding reduces the amount of abrasive retained on the dentin surface and prevents dust formation during the procedure.[28] The presence of debris on the abraded surface may interfere with the adhesive systems' chemical bond and micromechanical retention.[1,22,25,26,29]

Regarding PC-Bond group, a well-defined and homogenous adhesive interface with small resin tags is observed in a transversal view, while in a longitudinal perspective, an irregular surface with few discontinuity *loci* is represented. Souza-Zaroni *et al.* characterized the adhesive interface previously air abraded with Al_2O_3 and followed by the application of a self-etch adhesive and found hybrid layers with homogeneous thickness.[24]

Yazici *et al.* stated that the need for pretreatment of dentin prior to applying self-etch adhesives is somewhat controversial and defeats the original purpose of these systems.[20]

3. Air abrasion with bioactive glass

Air abrasion has been advocated to be a suitable method to prepare conservative cavities, selectively removing infected dentin.(14)

According to Paolinelis *et al.*, air abrasion with bioactive glass may be used to prepare both sound and carious dentin with a slower cutting rate when compared to aluminum oxide powder.(28)

The difference in the alumina and bioactive glass cutting rate is due to the brittle nature of the bioactive glass particles causing the particle itself to fracture on impact with harder sound dentine.(28) Although Bioglass 45S5 has Young's modulus of 35 GPa and Vickers hardness of 458 VHN, which is lower than alumina's (380 GPa and 2300 VHN, respectively), it is still similar to the hardness of mineralized dentin.(14)

However, literature is scarce and morphological changes following surface treatment with BAG and subsequent application of bonding agents remain unclarified.

Our findings indicate the presence of a thick and amorphous smear layer responsible for the occlusion of dentin tubules in *Sylc* group, crowded by micro-fissures branching from the dentinal tubules. These fissures disappear in the groups where smear layer was removed, thus leading to the belief that they are restricted to the aforementioned layer without affecting underlying dentin.

Previous studies demonstrate that pretreatment of dentin surface by means of BAG air abrasion may have the potential to induce remineralization within the dentin-bond interface, contributing to the inhibition of endogenous dentin proteases that cause degradation of collagen fibrils. According to some authors, BAG particles lead to a biomimetic process characterized by silicic acid release and a subsequent poly-condensation reaction. The presence of fluids analogous to saliva or body fluids (i.e., PBS) boosts an immediate exchange between sodium ions and hydrogen cations, inducing a rapid release of calcium ions and phosphate from the bioglass structure. An increase in pH seems to promote the precipitation of calcium and phosphate from the particles and from PBS to form an amorphous calcium phosphate layer that is subsequently hydrolyzed into hydroxyapatite.(17,18,30)

In *Sylc*-Primer group, CSE's primer dissolved the smear layer and opened the dentin tubules, similarly to PC-Primer group. BAG particles deposited on dentin's surface were observed at the sample's full length, but contrarily to Al_2O_3 groups, no particles were found embedded or pierced in the dentinal structure. Additionally, the presence of bioactive glass particles on dentin surface is consistent with a study by Carvalho *et al.* that reported the formation of a "bioactive smear layer" after air abrasion with an experimental bioactive glass and verified that dentin pretreatment with this powder did not interfere with the performance of restorative materials.[16]

Regarding *Sylc*-Bond group, interruption of the smooth CSE surface by cracks and crater-like *loci* seen throughout the longitudinal view suggests an interference between dentin and adhesive system might have occurred or simply that loose BAG particles deposited over the dentin may have been displaced, dragging chunks of adhesive along. Sauro *et al*. concluded that air abrasion procedures performed using pure BAG do not interfere with the bonding performance of self-etch systems formulated with functional monomers (10-MDP or 4-methacryloxy-ethyl-trimellitate), which are also in the composition of CSE.[14] This author also found that the combination of residual bioactive glass particles and hydroxyapatite results in remineralization and physical occlusion of dentin tubules.[18]

As mentioned before, air abrasion procedures were performed under a continuous spray of a water-based solution containing 17.5% ethanol. However, a recent study showed that using BAG combined with a polyacrylic acid fluid rather than BAG and water, might increase the probability of BAG embedding in dentin tubules.[17]

In what concerns clinical practice, Al_2O_3 and BAG air abrasion requires some precautions. In order to perform these procedures, rubber damn isolation and an adequate suction of the airborne particles cloud (powders and irrigant) are imperative to avoid ingestion or inhalation. Moreover, air abrasion standardization through maintaining steady parameterized angulation, distance, pressure and time, represent not only a clinical challenge but also a methodological uncertainty to this day, as literature is scarce and the few available studies are markedly heterogeneous.[31]

SEM sample examination confirmed that AquaCare powders and surface preparation strategies used in this study induced alterations in dentin morphology, thus confirming the initial hypothesis of this work.

4. Considerations and limitations

The purpose of this pilot study was to assess the effects of air abrasion with two AquaCare powders on dentin, before and after the application of CSE adhesive system. This study's limitations reside in the small number of samples included and that only a morphological characterization of samples was carried out.

Further studies should be conducted to evaluate the effects of surface treatment with AquaCare air abrasion unit on bond strength of different adhesive bonding strategies to dentin, with and without artificial aging, to predict the long-term clinical durability of resin-dentin bonding.

Conclusion

Studies assessing the effects of dentin surface pretreatment with air abrasion systems are still lacking. Moreover, the few available studies report contradictory results, thus making it challenging to render solid conclusions. Our findings indicate that dentinal surface treatment with AquaCare powders influences the type of smear layer and the adhesive interface obtained with CSE adhesive system. Further studies are crucial to formulate guidelines regarding the recommended parameters for dentin preparation, as well as to provide a better understanding of the effects of abrasive powders on dentin bonding.

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