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Characterization of dentin adhesion after air-abrasion with aluminum oxide particles: pilot study

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Summary

Resumo

Introdução: A obtenção de uma camada híbrida com elevada qualidade é o maior desafio na adesão dentinária, uma vez que é um fator crucial para garantir o sucesso a longo prazo das restaurações adesivas. O jateamento com óxido de alumínio consiste num tratamento mecânico que utiliza partículas de óxido de alumínio para introduzir modificações à superfície e tem sido utilizado como método de limpeza dentinário prévio aos procedimentos adesivos. O objetivo deste estudo é avaliar o efeito do jateamento com óxido de alumínio nas forças de adesão à dentina de diferentes sistemas adesivos.

Materiais e Métodos: A porção de esmalte oclusal de 6 molares humanos extraídos foi seccionada e a superfície de dentina lisa resultante foi tratada com uma sequência de lixas de água para criação de uma *smear-layer* padronizada. Os dentes foram aleatoriamente alocados em 6 grupos experimentais, de acordo com tratamento de superfície e sistema adesivo: G1: Jateamento + Clearfil™ SE Bond; G2: Jateamento + Optibond™ FL; G3: Jateamento + ScotchBond™ Universal; G4: Clearfil™ SE Bond; G5: Optibond™ FL; G6: ScochBond™ Universal. Depois do procedimento adesivo, realizou-se um *build-up* de 5mm em resina composta, armazenando-se posteriormente em água destilada. As amostras foram seccionadas em dois eixos por forma a obter *sticks* com área média de 1.41 mm^2, testados numa máquina universal de testes. Dois espécimes por grupo foram preparados para microscopia eletrónica de varrimento.

Resultados: Two-way ANOVA não detetou diferenças estatisticamente significativas entre os vários sistemas adesivos $F(2, 83)=2,548$, $p=0.084$. O jateamento da dentina produziu um decréscimo significativo na força de adesão $F(1, 83)=11.04$, $p=0.001$, reduzindo-a, em média, em 7.44 MPa (95%CI:[2.99;11.89]).

Conclusões: Tendo em consideração as limitações deste estudo, verificou-se que o jateamento da dentina com óxido de alumínio afetou negativamente a interação entre os sistemas adesivos e o substrato dentinário, diminuindo as forças de adesão.

Palavras-chave: Dentina; Jateamento; Óxido de alumínio; Força de adesão; Microscopia eletrónica de varrimento.

Abstract

Introduction: Achieving a reliable hybrid layer is the main challenge of dentinal adhesion, as this is a major factor to ensure the long-term success of adhesive restorations. Aluminum oxide air abrasion consists in a mechanical treatment that uses aluminum oxide particles to introduce surface modifications and has been used as a dentin surface cleansing method prior to adhesive procedures. The aim of this study is to evaluate the effect of aluminum oxide air abrasion on the bond strength of different adhesive systems to dentin.

Materials and Methods: Flat dentin surfaces were prepared in 6 extracted human molars. Exposed dentin surfaces were abraded with a sequence of silicon carbide sandpaper to create a standardized smear layer. The teeth were randomly allocated into 6 experimental groups according to the dentin pretreatment and adhesive system tested: G1: Sandblasting + Clearfil™ SE Bond; G2: Sandblasting + Optibond™ FL; G3: Sandblasting + ScotchBond™ Universal; G4: Clearfil™ SE Bond; G5: Optibond™ FL; G6: ScochBond™ Universal. After adhesive procedures, a 5 mm thick composite crown was built over the bonded surface. Following the storage in distilled water, the samples were vertically cross-sectioned until obtaining sticks with 1.41 mm^2 of cross-sectional area, which were tested using a universal testing machine. Two specimens of each group were collected for analysis by scanning electron microscopy.

Results: Two-way ANOVA did not detect statistically significant differences among adhesive systems $F(2, 83)=2,548$, $p=0.084$. Al_2O_3 sandblasting produced a significant decrease in μTBS $F(1, 83)=11.04$, $p=0.001$, reducing the bond strength in 7.44 MPa (95%CI:[2.99;11.89]).

Conclusions: Within the limitations of this pilot study, it may be concluded that dentin pre-treatment with Al_2O_3 adversely affected the interaction pattern between adhesive systems and dentin substrate, decreasing microtensile bond strength.

Keywords: Dentin; Sandblasting; Aluminium Oxide; Microtensile Bond Strength; Scanning Electron Microscopy

Introduction

The main challenge of adhesion to dentinal substrates is creating a reliable hybrid layer, as this consists the main critical factor for the success and longevity of adhesive restorations (1-5). This process, called hybridization, is based in a superficial demineralization followed by resin monomers infiltration that upon setting become micro-mechanically interlocked to dentin surface (2, 6, 7). The infiltration of these adhesive monomers may be impaired, as described in previous studies, by the presence of smear layer that may constitute a physical barrier (2, 7). Therefore, dentin surface treatments for smear layer cleaning, such as its complete removal, dissolution, replacement or modification, should be considered as a decisive step previous to restoration bonding procedures (2-4, 8, 9)

These surface modifications are often part of the adhesive systems responsible for bonding restorative materials to tooth structure, and according to their distinct hybridization techniques a simple division between two groups: etch-and-rinse and self-etch adhesives may be established (2, 3).

Etch-and-rinse adhesive systems can be applied in three or two steps, always requiring an initial acid-etching phase of the dentin substrate (2, 5-7). In three-step systems, the gold-standard of etch-and-rinse adhesives, the acid etching is followed by the intermediate application of a primer, ending with the application of a hydrophobic resin (10, 11). The primer, composed by amphipathic monomers dissolved in organic solvents (acetone, alcohol and/or water), aims to alter the hydrophilic dentin surface into a hydrophobic phase, ensuring an increase on the dentin surface energy, thus this substrate becomes an adequate receptor of the hydrophobic bonding resin (10). Amphipathic monomers have hydrophilic properties with affinity for the exposed collagen fibrils and hydrophobic features, enabling co-polymerization with the adhesive resin (10). The hydroxyl ethyl methacrylate (HEMA) monomer, which has the capacity to potentiate re-expansion of the collagen mesh, is the most frequently monomer incorporated in primers (10). The hydrophobic resin enables the micromechanical retention by hybridizing the intertubular dentin and tubule walls, through its diffusion into the interfibrillar collagen channels (2, 10). For the two-step systems, primer and resin are combined in a single bottle (3, 10, 11). Dentin etching with phosphoric acid leads to a complete removal of the smear layer, demineralizing the intertubular dentin, thus leaving a tridimensional collagen mesh exposed, practically devoided of hydroxyapatite, that should be able to mechanically retain the resin monomers upon *in situ* polymerization,

enabling the formation of a mixed structure, the hybrid layer (2, 5-7). This technique is considered to be critical and highly sensitive, creating issues with the wet-bonding protocol, because unintentional over-drying of dentin after acid etchant rinsing considerably increases the risk of collapse of the demineralized collagen mesh and lowers monomer diffusion throughout the intertubular dentin (3, 6-8, 11). On the other hand, in over-wet dentin, there seems to exist a phase separation between the hydrophilic and hydrophobic components of the adhesive, leading to incomplete monomer polymerization and adsorption of water in the hybrid layer, thus making the adhesive interface vulnerable to the degradation process (3, 6-8, 11).

Later, another category of adhesive systems was conceived with the purpose of avoiding the presence of unprotected collagen fibrils and subsequent adhesion failure, causing a lesser degree of demineralization and promoting a more complete bond between tooth structure and restorative material (6-8). Self-etching adhesives contain non-rinse acidic monomers that dissolve the smear layer, eliminating the acid conditioning step and thus decreasing the risk of collagen network collapse, since the carboxylate or phosphate acidic groups simultaneously etch and prime the dentin substrate. This technique also exhibits reduced sensitivity, as the level of dentin moistness is no longer a critical issue to the bonding procedure (2, 3, 5, 6, 8, 12).

Self-etch adhesives are classified as two-step and one-step systems. The two-step systems are based on the application of a hydrophilic self-etching primer followed by a hydrophobic bonding resin, representing the gold standard of self-etch adhesives (10, 11). The primers are formed by aqueous mixtures of acidic functional monomers, generally phosphoric acid- or carboxylic acid-esters, such as 10-Methacryloyloxydecyl dihydrogen phosphate (10-MDP), that are able to create ionic bond between their functional groups and hydroxyapatite calcium (10). Self-etching primers composition is unique, since they contain high concentrations of water, acting as an ionizing vehicle for dissociation of acidic protons and dissolution of calcium ions, promoting the surface etching, while simultaneously preventing the collapse of collagen network (10). Co-solvents, such as acetone or ethanol, are frequently added to form an azeotropic solution with water, thus promoting solvation of hydrophobic components and ensuring proper dentin wettability (10). One-step systems consist in a complex mixture of hydrophilic and hydrophobic components, however the water present in this “all-in-one” adhesives might cause a degradation of these systems, leading to a phase separation of the monomers. In addition, it might be difficult to completely evaporate the water during the air-drying step, leading to an incomplete adhesive polymerization and increased hydrolysis, thus compromising the bonding durability (10). In addition, several characteristics of the

different self-etching adhesives are attributed to the reported variability of performances, particularly, some properties of the functional monomers, such as its acidity (10). The acidity and aggressiveness of the self-etch adhesive systems are established by the concentration and composition of acidic resin monomers, determining their effect on smear layer dissolution and demineralization capacity. Therefore, aggressiveness is correlated to the pH of self-etching systems and these can be categorized as “ultra-mild” (pH higher than 2.5), “mild” (pH around 2), “intermediately strong” (pH between 1 and 2) and “strong” (pH lower than 1) (10).

It remains unclear which adhesive strategy achieves greater adhesion strength, yet the choice must be based in the substrate nature and technique sensitivity, as an adequate result may be impaired by the impossibility of performing a correct technique (13). Apart from technical considerations, enamel and dentin have a quite distinct composition (6). Enamel has a mainly inorganic phase, hydroxyapatite, with a low organic and water content, while dentin is intrinsically humid and has higher organic content, undergoing changes with the aging process. In addition, contrarily to dentin, enamel can be dried easily, effortlessly and with no concerns for its structural integrity, leading to the necessity of different adhesion strategies for each type of tooth structure (14, 15).

Considering the disparity in professional judgement regarding the selection of the adhesive system, manufacturers have released a new generation of adhesives (2, 3, 7). These new materials are known as “universal”, “multi-mode” or “multipurpose” and, due to their versatility, can be applied both with the etch-and-rinse or self-etch approach, allowing the practitioner to decide which protocol is more suitable for each clinical situation (2, 3, 7). Regarding dentin, and considering that there is no evident increase in bond strength when acid conditioning is performed, in “mild” (pH around 2) universal adhesives a self-etch protocol must be followed, leading to a reduced risk of post-operative sensitivity (2). These one-bottle adhesive systems present a challenge, as far as their chemical formula is concerned, since a balance between hydrophilic and hydrophobic character is necessary, as the monomers need to be hydrophilic enough to wet, infiltrate and interact with the dentin substrate. However after polymerization, hydrophilicity promote water sorption, leading to hydrolysis and degradation of the adhesive interface (10, 16). Universal adhesives manufacturers’ approach these issues combining hydrophobic monomers, such as bisphenol A diglycidylmethacrylate (bis-GMA) and hydrophilic monomers, such as HEMA, along with adhesive functional monomers (10, 16). Almost every adhesive systems include HEMA in their formulations, not only to ensure dentin wettability, but also for their solvent-like properties (2, 3, 16). Despite all of the advantages, HEMA present some negative aspects, since in both

uncured and polymerized state promptly absorbs water, and once polymerized it can discolour, swell and contribute to hydrolysis of the bonding interface (16). Aiming to increase surface roughness in dental hard tissues and ceramic or composite restorations, several strategies have been suggested, being the airborne-particle abrasion one of the most widely used nowadays for the preparation of conservative cavity designs and removal of decayed tissue (1, 3, 6, 8, 14, 15, 17-20).

Aluminium oxide air abrasion consists in a mechanical treatment that uses Al_2O_3 to introduce surface changes and was recently suggested as a dentin surface cleaning method (1, 5, 6, 14, 15, 20). As the particles reach the dentin, the kinetic energy is released, resulting in microscopic fractures of the surface (4, 5, 11, 18, 19, 21). This is hypothesised to allow an enhancement of mechanical interlocking between resin monomers and intertubular dentin, promoting higher bond strength values (1, 17). The aim adjacent to this strategy is to create an uneven surface, enlarging the available area for adhesion, and thus obtaining an increased wettability of adhesive systems to tooth structure (1, 3, 4, 17-20).

As a disadvantage, it is thought that the permanence of the Al_2O_3 debris on the dentin surface may influence the penetration of resin monomers, presenting a risk for the adhesion success (6). In spite of the possibility to take the powder cloud generated during sandblasting as potentially dangerous for both the dentist and the patient, it has been demonstrated that the amount of dust that is produced does not represent a hazard for human health and can be easily controlled with adequate suction (22).

The aim of this study is to evaluate the effect of aluminium oxide air abrasion on the bond strength of different adhesive systems to dentin. The null hypothesis tested in this study is that (H_0) no differences in dentin microtensile bond strength are found when dentin suffers no treatment or is sandblasted with aluminium oxide; and (H_1) no interaction effect occurs between sandblasting and adhesive systems.

Materials and Methods

Specimen Selection and Preparation

Six human molars were extracted and kept in distilled water. The teeth were cleaned from debris, access to the pulp chamber was performed through the root furcation with a diamond bur in high speed turbine, and after applying a universal adhesive system in self-etch mode (One Coat 7 Universal, COLTENE, Switzerland – lot H61348, expiration date 2019-04), pulp chambers were filled with a dual curing resin cement (DuoCem®, COLTENE, Switzerland – lot H01432, expiration date 2018-05). A silicone putty matrix (PRESIDENT The Original® Putty Soft, COLTENE, Switzerland) was used for the partial inclusion of the roots in acrylic resin blocks (ProBase® Cold, Ivoclar Vivadent, Lichenstein). The occlusal surfaces were cut perpendicularly to the long axis of the tooth with a precision cut-off machine (Accutom-5, Struers, USA), with integrated cooling system. The flat dentin surfaces were abraded with a sequence of 120-220-600-grit silicon carbide sandpaper in a circular motion for 60 seconds to create a standardized smear layer.

Experimental Protocol and Dentin Pretreatment

The specimens were randomly assigned into 6 experimental groups by a computer algorithm (www.randomizer.org; Urbaniak, G. C., & Plous, S. (2013); Research Randomizer, Version 4.0) , accordingly to surface treatment and adhesive system.

Group 1: dentin with smear layer abraded with Al_2O_3 particles + adhesive system Clearfil™ SE Bond

Group 2: dentin with smear layer abraded with Al_2O_3 particles + adhesive system Optibond™ FL

Group 3: dentin with smear layer abraded with Al_2O_3 particles + adhesive system ScotchBond™ Universal

Group 4: dentin with smear layer + adhesive system Clearfil™ SE Bond

Group 5: dentin with smear layer + adhesive system Optibond™ FL

Group 6: dentin with smear layer + adhesive system ScochBond™ Universal

The tip of a Airsonic® Mini Sandblaster (Hager & Werken) was positioned 1 cm from dentin surface, and a gutta-percha cone was used to standardize this distance. Abrasion of specimen surfaces was performed using aluminium oxide particles of 50 µm, with 2.0 bar pressure, for 6 seconds.

Adhesive systems were applied according to manufacturers' instructions. For Clearfil™ SE Bond, a two-step self-etch adhesive system, primer was applied using a microbrush for 20 seconds, surface was gently air dried to evaporate the solvent, adhesive resin was applied and air stream was used to remove the excesses until a shiny and steady layer was obtained, followed by 20 seconds of light curing (Bluephase Style 20i®, Ivoclar Vivadent, Lichenstein).

For Optibond™ FL, a three-step total-etch adhesive system, 37% phosphoric acid was applied to the dentin surface for 15 seconds, the surface was rinsed until etchant has been completely removed and excess water was absorbed. Primer was actively applied using a microbrush for 15 seconds and then gently air dried for approximately 5 seconds. Adhesive resin was applied and air was used to remove the excesses until a shiny and steady layer was obtained, followed by 20 seconds of light curing (Bluephase Style 20i®, Ivoclar Vivadent, Lichenstein).

For Scotchbond™ Universal, the adhesive was actively applied using a microbrush for 20 seconds and then gently air dried for 5 seconds to evaporate the solvent, followed by a 20 seconds light curing (Bluephase Style 20i®, Ivoclar Vivadent, Lichenstein).

Following application of the adhesive systems, a build-up with nano-hybrid composite (Tetric EvoCeram®, Ivoclar Vivadent – lot W93406, expiration date 2021-10-19) was performed. Each layer was polymerized for 20 seconds followed by an extra-time final light curing of 60 seconds (Bluephase Style 20i®, Ivoclar Vivadent, Lichenstein). After curing, the teeth were stored in distilled water at room temperature during a week.

Table I: Adhesive systems studied, manufacturers, lot and expiration date, chemical composition, group and surface treatment, and application mode.

Adhesive System Manufacturer Lot/Exp	Composition	Group: Surface Treatment	Application Mode
Clearfil™ SE Bond Kuraray Medical, Tokyo, Japan 840034 2018-12	Primer: 10-MDP, HEMA, dl-camphorquinone, hydrophilic aliphatic dimethacrylate, N,N-Diethanol-p-toluidine, water. Bond: 10-MDP, BisGMA, HEMA, dl-camphorquinone, colloidal silica, N,N-Diethanol-p-toluidine, hydrophobic aliphatic dimethacrylate	G1: SB Al_2O_3 (Al_2O_3 + CSE)	Dentin sandblasting 6 seconds; Apply primer 20 seconds; dry with mild air flow; apply bond; air flow gently; light-cure 20 seconds
		G4: No (CSE)	Apply primer 20 seconds; dry with mild air flow; apply bond; air flow gently; light-cure 20 seconds
Optibond™ FL Kerr, Orange, CA, USA 6394161 2018-11	Primer: HEMA, GPDM, PAMM, ethanol, camphorquinone, water. Bond: BisGMA, HEMA, GPDM, camphorquinone, glycerol, barium aluminoborosilicate, silicon dioxide dimethacrylate resins	G2: SB Al_2O_3 (Al_2O_3 + OFL)	Dentin sandblasting 6 seconds; apply 37% phosphoric acid 15 seconds; rinse and absorb excess water; actively apply primer 15 seconds; air-dry 5 seconds; apply bond; air flow gently; light-cure 20 seconds
		G5: No (OFL)	Apply 37% phosphoric acid 15 seconds; rinse and absorb excess water; actively apply primer 15 seconds; air-dry 5 seconds; apply bond; air flow gently; light-cure 20 seconds
Scotchbond™ Universal 3M ESPE, St Paul, MN, USA 635451 2018-07	HEMA, dimethacrylate resins, 10-MDP, Vitrebond™ copolymer, filler, ethanol, water, initiators, silane	G3: SB Al_2O_3 (Al_2O_3 + SBU)	Dentin sandblasting 6 seconds; Actively apply for 20 seconds; dry with mild air flow; light-cure 20 seconds
		G6: No (SBU)	Actively apply for 20 seconds; dry with mild air flow; light-cure 20 seconds

CSE – Clearfil™ SE Bond; **OFL –** Optibond™ FL; **SBU –** Scotchbond™ Universal; **Al_2O_3** – dentin sandblasting with aluminum oxide; **MDP** - 10-Methacryloyloxydecyl dihydrogen phosphate;

HEMA - 2-hydroxyethyl methacrylate; **BisGMA** - bisphenol A diglycidylmethacrylate; **GPDM** - glycerol phosphate dimethacrylate; **PAMM** - phthalic acid monomethacrylate; **Vitrebond™ copolymer** – polyalkenoic acid copolymer

Cutting Method

The specimens were cross-sectioned perpendicularly to the adhesive-tooth interface with a low-speed cutting saw in a precision cut-off machine (Accutom-5, Struers, USA), under water cooling, with a speed of 1000 rpm at 0.100 mm/s to produce dentin-composite resin sticks with a sectional square area of approximately 1.41 mm^2. After the first cut in x-axis direction, the free residual space between the slices was filled with light body silicone (Aquasil Ultra XLV, DENTSPLY Caulk, USA – lot 170616, expiration date 2020-06-28). After the cut in y-axis direction, the roots were cut from the crown approximately 2 mm bellow the cementoenamel junction releasing the dentin-composite sticks which were then checked under an optical microscope (Leica EZ4 HD, Switzerland) at 30-fold magnification in order to exclude samples with defects and residual enamel. Each stick was measured with a thickness gauge (Mitutoyo Digital Caliper, Japan) for later calculation of the adhesive interface area.

Microtensile Bond Strength Testing

Each stick was bonded to a microtensile sample holder with cyanoacrylate rubber enhanced superglue gel (CE10 Flex, Ce Chem Limited, UK – lot 3865, expiration date 2018-07) and then fixed on the microtensile device (Od04-Plus; Odeme Dental Research, Luzerna, Brasil). Specimens were fractured in tensile mode in a universal testing machine (Autograph®, Model AG-I, Shimadzu Corporation, Kyoto, Japan) at a 5 mm/min speed and the maximum load in Newton (N) at failure was recorded.

After microtensile testing, the fractured sticks were examined with a microscope (Leica EZ4 HD, Switzerland) at a 30-fold magnification and the failure mode was identified. Failure types were categorised as: (A) adhesive (total failure occurred within the adhesive interface); (CD) cohesive in dentin (complete failure in dentin); (CR) cohesive in composite (complete failure occurred in the composite resin) and, (M) mixed, when simultaneously the adhesive and cohesive failure occurred.

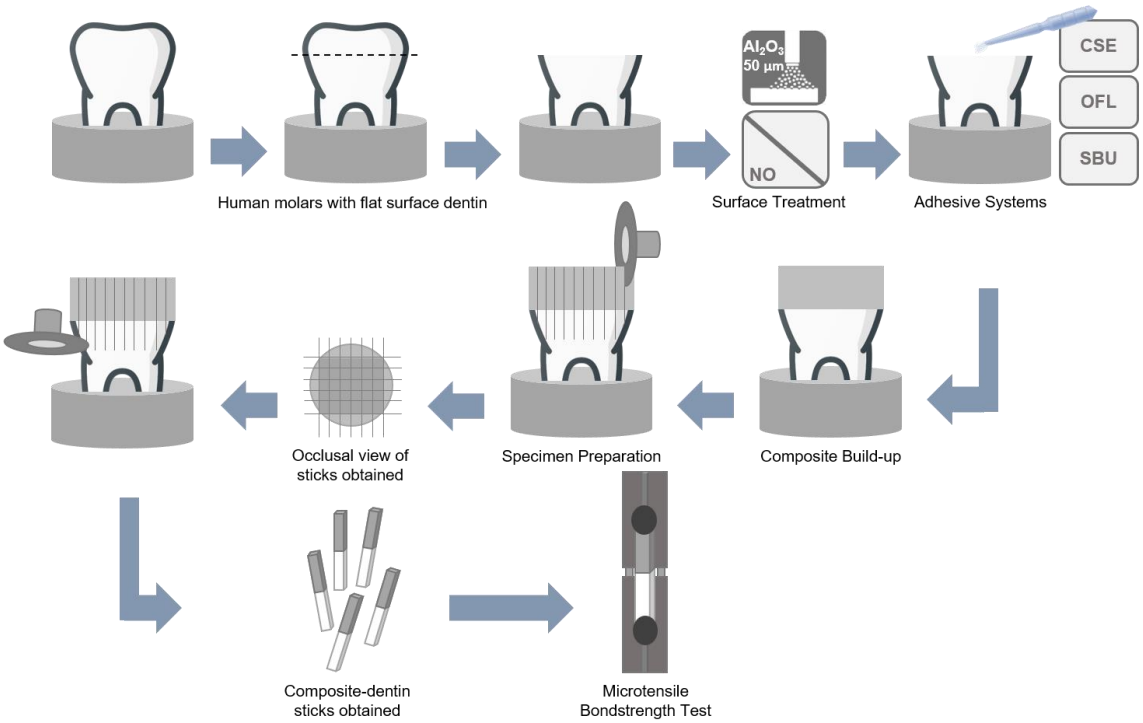

Figure 1: Schematic diagram from specimen preparation and microtensile bond strength test. **Al_2O_3** – sandblasting with aluminum oxide 50 µm; **CSE** – Clearfil™ SE Bond; **OFL** – Optibond™ FL; **SBU** – Scotchbond™ Universal

Ultra morphology Analysis by Scanning Electron Microscopy (SEM)

Two extra sticks of each group were collected for SEM ultra morphology analysis. The samples were immersed in hydrochloric acid for 30 seconds to achieve demineralization, followed by deproteinization with sodium hypochlorite for 10 minutes. Samples were rinsed with distilled water and were dehydrated in ascending ethanol series of 50%, 75%, 95% and 100% for at least 10 minutes per step, except the last one which was done for 16 hours.

After chemical dehydration, the specimens were mounted on a specimen aluminium stub using carbon adhesive, sputter-coated with gold-palladium (Polaron E5000 Sputter-Coater, Polaron Equipment Limited, Watford, UK) before SEM analysis with a Hitachi S-4100 microscope (Hitachi, Tokyo, Japan), in various magnifications (1000x, 2500x, 5000x).

Statistical Analysis

Statistical analysis was performed with the IBM SPSS 23.0® program (SPSS Inc., Chicago, IL, USA). The results were statistically analysed using two-way analysis of variance ANOVA, after confirming normal distribution of the results with Saphiro-Wilk statistical test. Comparisons between groups were done using the Bonferroni correction. The significance level was set at $\alpha=0.05$.

Results

Microtensile Bond Strength

A total of 90 specimens were available for microtensile testing. Fifteen sticks (n=15) were tested for each group. Figure 2 and Table II show the microtensile bond strength results (μTBS) for the three adhesive systems tested with and without dentin sandblasting as pre-treatment.

The Shapiro-Wilk test revealed that all experimental groups respect normality (p>0.05). It was also verified the homogeneity of variances by Levene's Test (p>0.05).

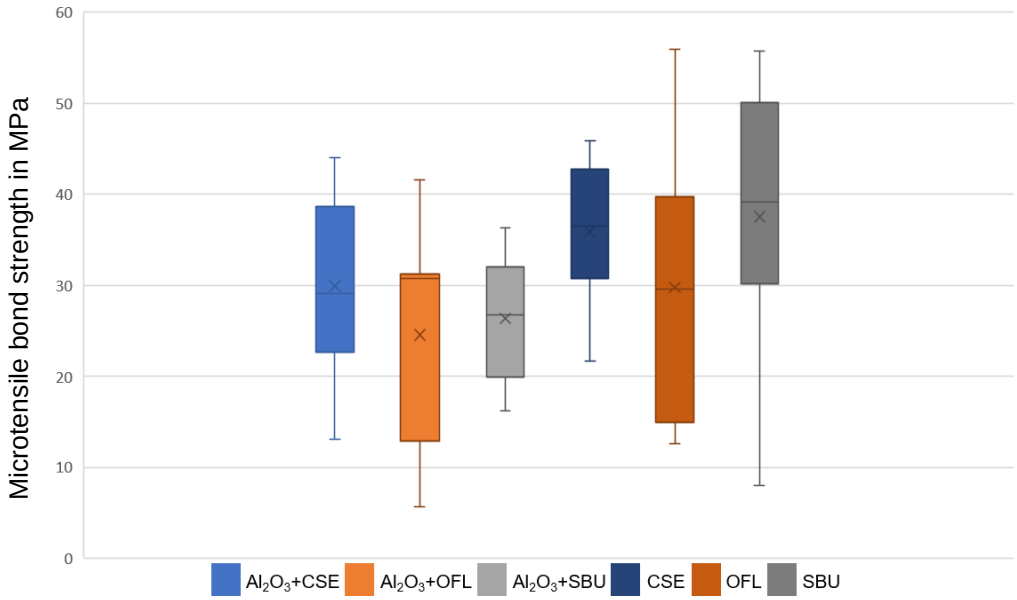

Figure 2: Box plot graphic for microtensile bond strength values distribution within groups.

Table II: Microtensile bond strength test results, mean and standard deviation, in MPa.

	CSE	OFL	SBU	p
Control	35.90 ± 7.69	29.76 ± 12.58	37.52 ± 14.60	0.189
Al_2O_3	29.93 ± 9.31	24.56 ± 10.55	26.38 ± 6.85	0.265
Total	32.91 ± 8.92	27.16 ± 11.71	31.75 ± 12.43	0.084

Mean adhesion values for the six experimental groups were compared using two-way ANOVA setting the significance level at α=0.05. ANOVA did not detect statistically

significant differences among adhesive systems F(2, 83)=2,548, p=0.084. Al_2O_3 sandblasting produced a decrease in μTBS F(1, 83)=11.04, p=0.001, decreasing the bond strength in 7.44 MPa (95%CI:[2.99;11.89]), as can be observed in Figure 3. There is no interaction between the type of the adhesive system and Al_2O_3 air abrasion, which means that sandblasting has the same effect in the three adhesive systems used F(2, 83)=0.685, p=0.507.

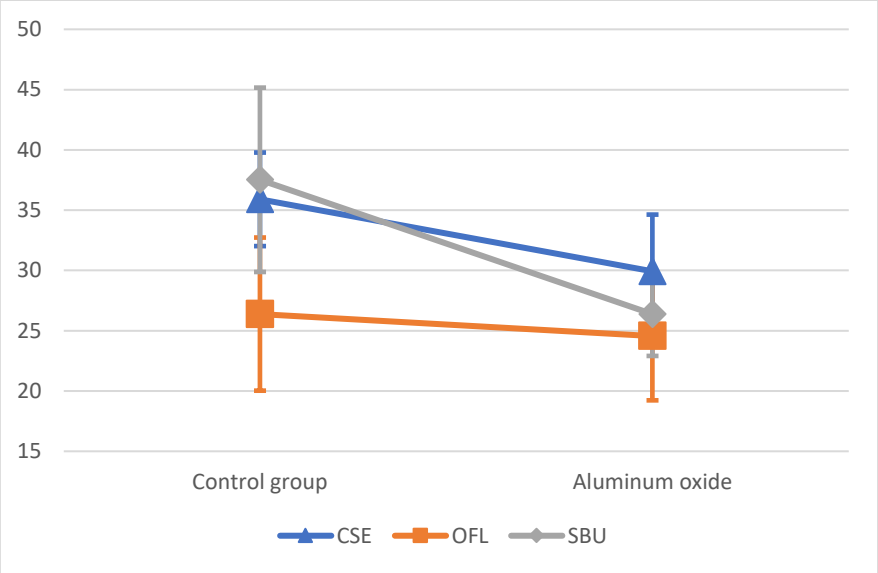

Figure 3: Mean values of the different adhesive systems, in MPa

Failure modes percentages are described in Table III and Figure 4.

Table III: Failure type results after microtensile bond strength test

	Group 1 (%)	Group 2 (%)	Group 3 (%)	Group 4 (%)	Group 5 (%)	Group 6 (%)
Adhesive	53%	60%	53%	40%	60%	43%
Cohesive in resin	47%	27%	20%	47%	40%	50%
Cohesive in dentin	0%	0%	0%	13%	0%	0%
Mixed	0%	13%	27%	0%	0%	7%

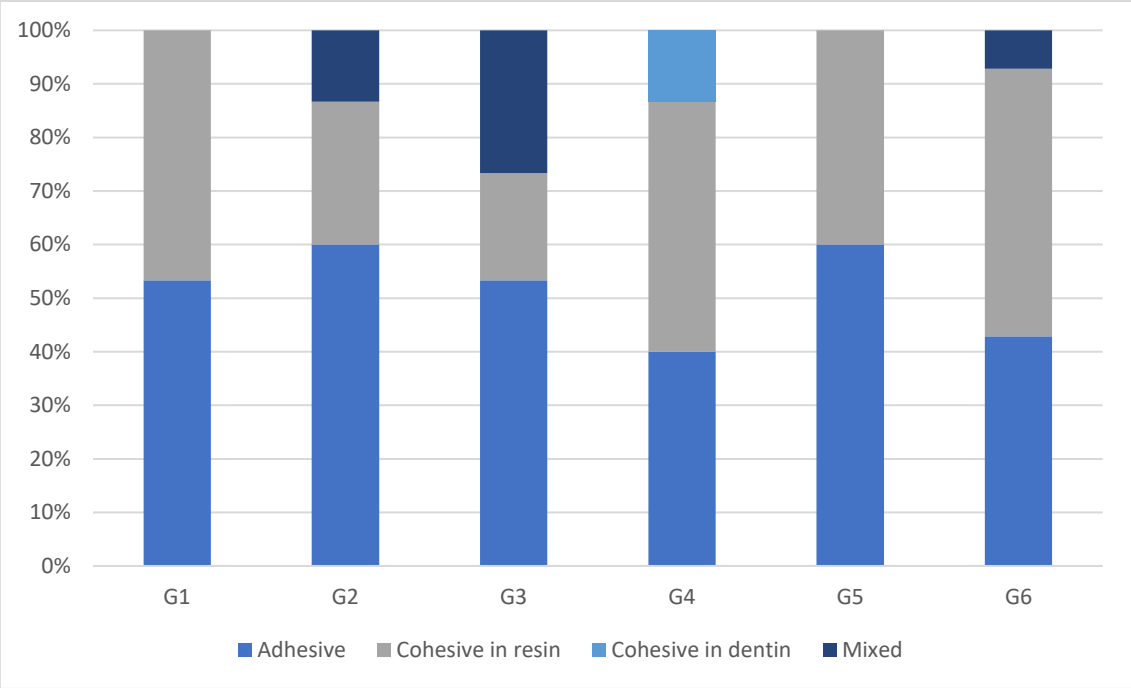

Figure 4: Failure types results after microtensile bond strength test

SEM observations

Representative micrographs of group 1, corresponding to Al_2O_3 sandblasted dentin and Clearfil™ SE Bond can be observed in figures 5, 7 and 9, at 1000, 2500 and 5000x magnification. Group 4, corresponding to dentin without Al_2O_3 air abrasion, where the self-etch adhesive was used, is represented in figures 6, 8 and 10 at 1000, 2500 and 5000x magnification.

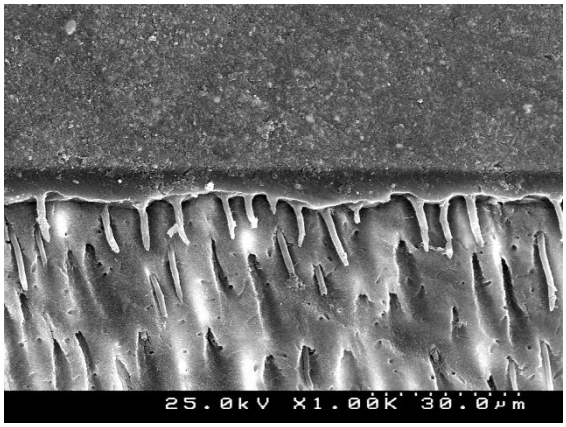

Figure 5: SEM image of adhesive interface of group 1 (Al_2O_3 + CSE) (1000x)

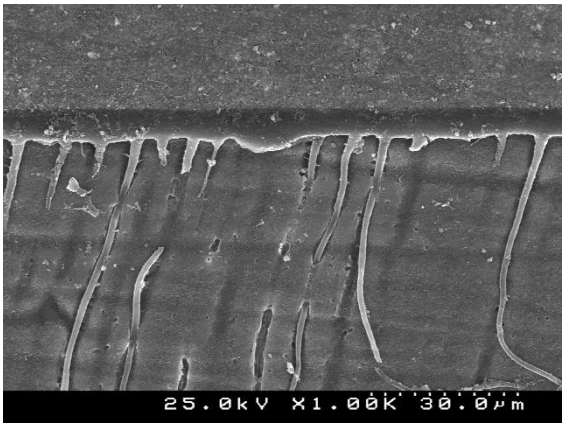

Figure 6: SEM image of adhesive interface of group 4 (CSE) (1000x)

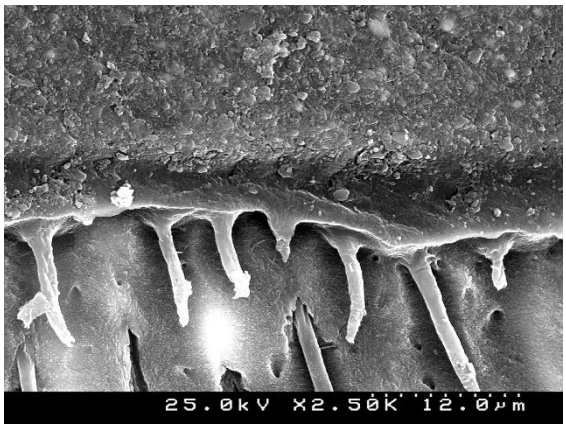

Figure 7: SEM image of adhesive interface of group 1 (Al_2O_3 + CSE) (2500x)

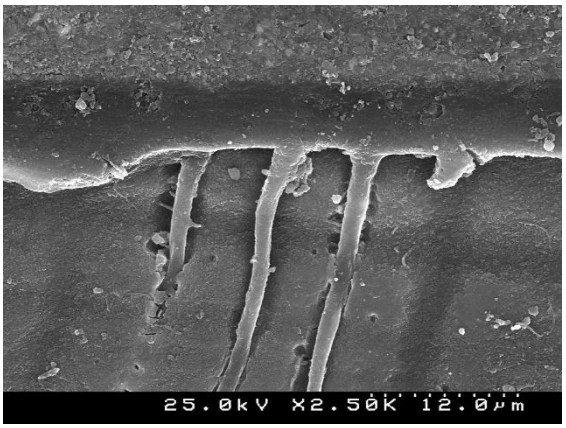

Figure 8: SEM image of adhesive interface of group 4 (CSE) (2500x)

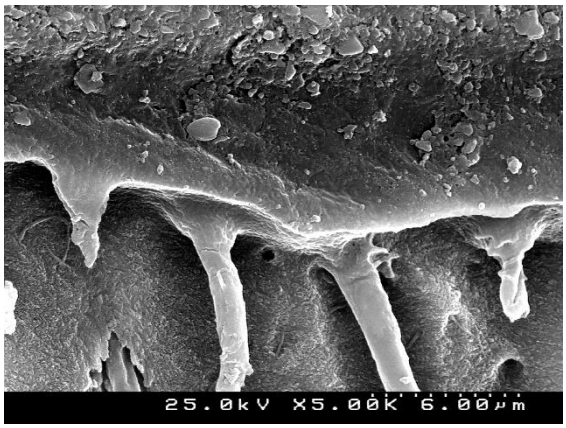

Figure 9: SEM image of adhesive interface of group 1 (Al_2O_3 + CSE) (5000x)

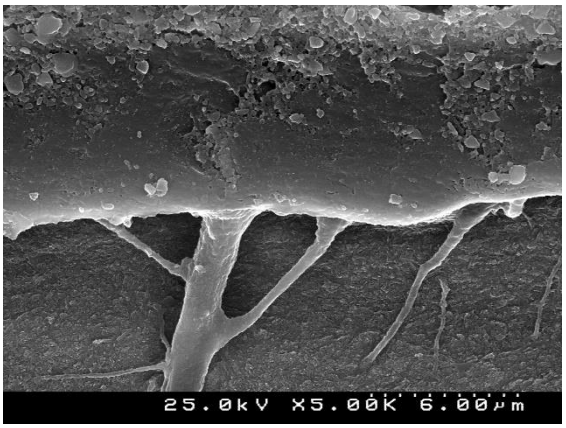

Figure 10: SEM image of adhesive interface of group 4 (CSE) (5000x)

In the micrographs of group 1, a thick layer of the adhesive and a inhomogeneous link between dentin and the adhesive are observed. Group 4 presents a more homogeneous and well-defined interface between dentin and the adhesive, suggesting better dentin hybridization.

Figures 11, 13 and 15 represent the micrographs of group 2, corresponding to Al_2O_3 sandblasted dentin and Optibond^{TM} FL, at 1000, 2500 and 5000x magnification. Group 5, corresponding to dentin without Al_2O_3 air abrasion, where the etch-and-rinse adhesive was used, is represented in figures 12, 14 and 16 at 1000, 2500 and 5000x magnification.

The micrographs of group 2 present a more irregular and less well-defined hybrid layer, furthermore the dentinal tubules orifices present the original diameter. In group 5, the hybrid layer is well-defined, moreover is possible to observe a widening in the coronal portion of the dentinal tubules.

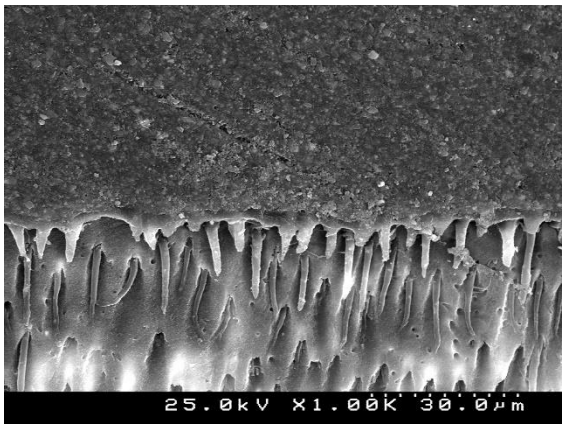

Figure 11: SEM image of adhesive interface of group 2 (Al_2O_3 + OFL) (1000x)

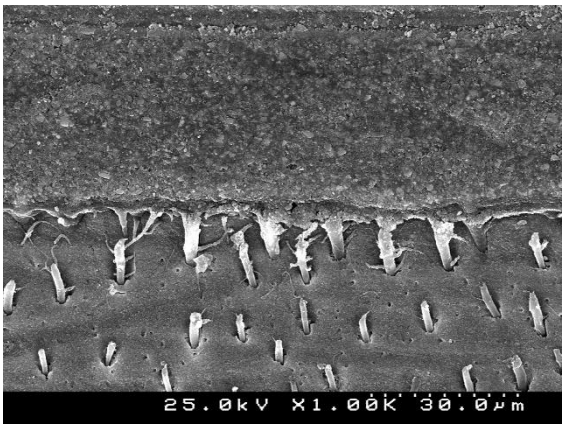

Figure 12: SEM image of adhesive interface of group 5 (OFL) (1000x)

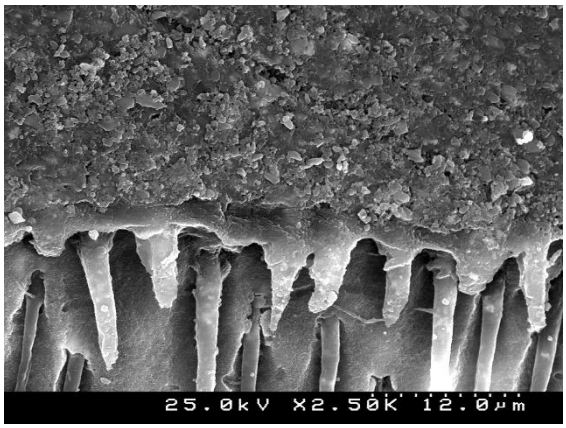

Figure 13: SEM image of adhesive interface of group 2 (Al_2O_3 + OFL) (2500x)

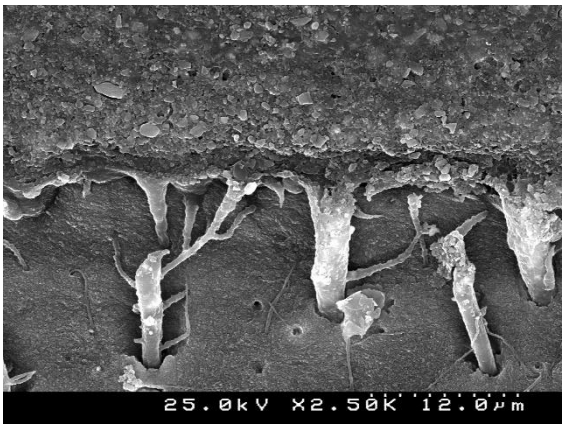

Figure 14: SEM image of adhesive interface of group 5 (OFL) (2500x)

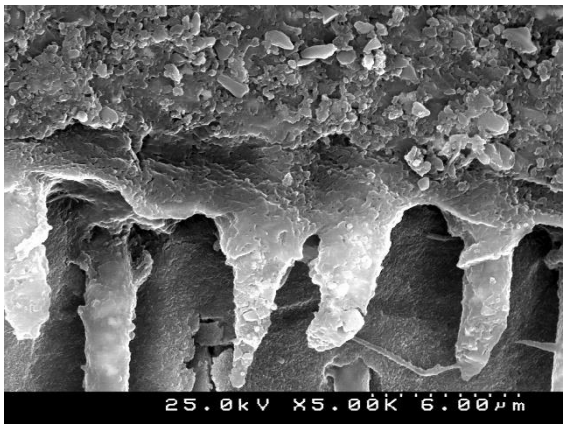

Figure 15: SEM image of adhesive interface of group 2 (Al_2O_3 + OFL) (5000x)

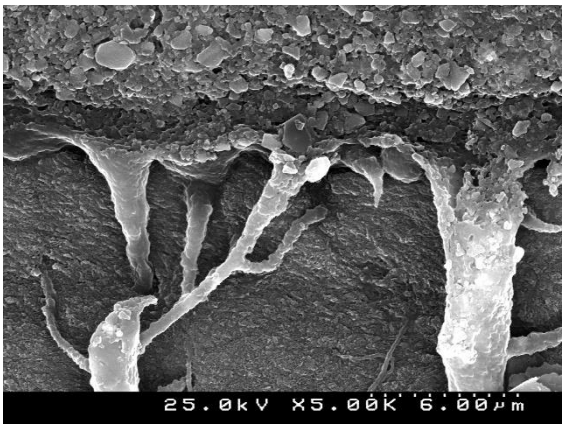

Figure 16: SEM image of adhesive interface of group 5 (OFL) (5000x)

The representative micrographs of group 3, corresponding to Al_2O_3 sandblasted dentin and Scotchbond™ Universal can be observed in the figures 17, 19 and 21 at 1000, 2500 and 5000x magnification. Group 6, corresponding to dentin without Al_2O_3 air abrasion,

where universal adhesive was used, is represented in figures 18, 20 and 21 at 1000, 2500 and 5000x magnification.

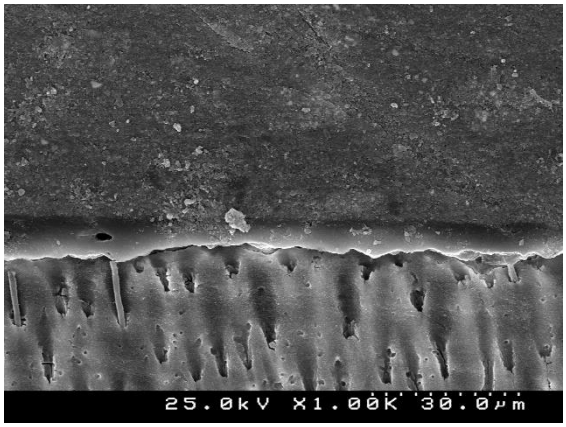

Figure 17: SEM image of adhesive interface of group 3 (Al_2O_3 + SBU) (1000x)

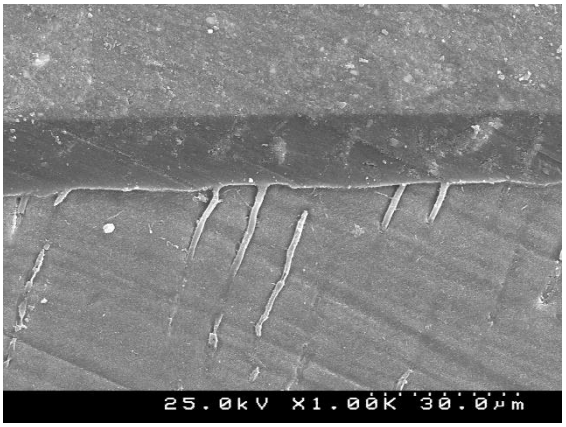

Figure 18: SEM image of adhesive interface of group 6 (SBU) (1000x)

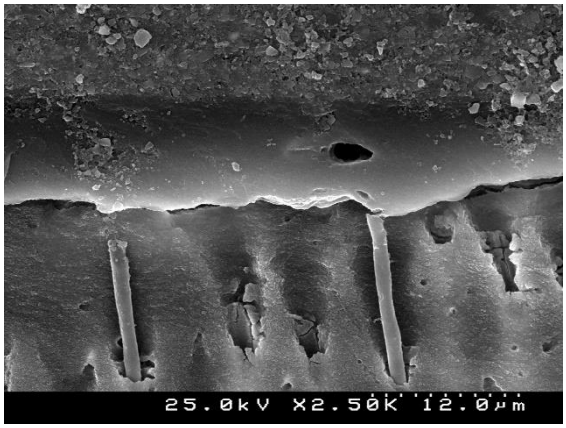

Figure 19: SEM image of adhesive interface of group 3 (Al_2O_3 +SBU) (2500x)

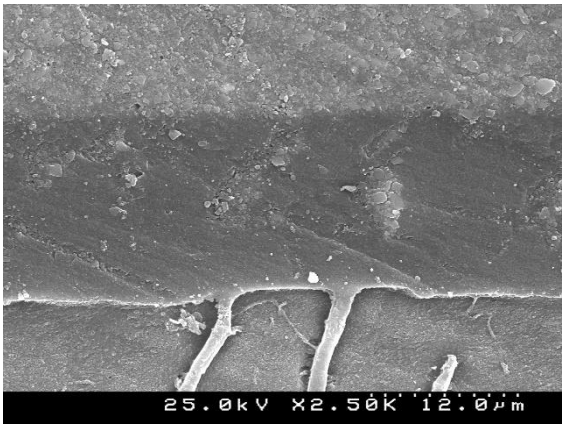

Figure 20: SEM image of adhesive interface of group 6 (SBU) (2500x)

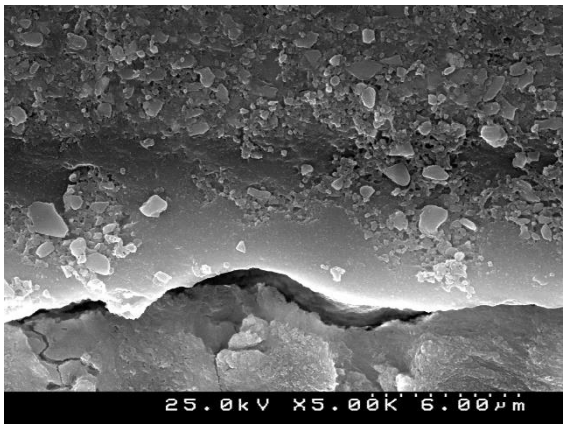

Figure 21: SEM image of adhesive interface of group 3 (Al_2O_3 + SBU) (5000x)

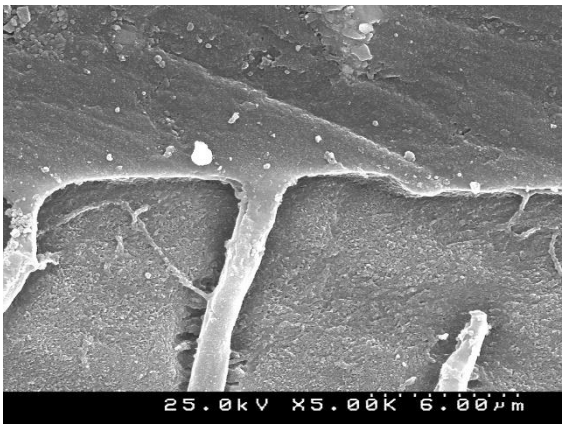

Figure 22: SEM image of adhesive interface of group 6 (SBU) (5000x)

In group 3 micrographs it is possible to observe a noticeable separation between adhesive system and dentin, with scarcely any resin tags. In the micrographs of group 6, a more homogeneous adhesive interface is observable, presenting longer resin tags and in higher number.

Discussion

Despite the advancements of modern dentistry, the ability to provide an equally effective adhesion to dentin when compared to enamel, remains a challenge (2, 19). Due to the nature of dentin substrate, namely its morphologic characteristics, higher organic content, the presence of fluid in dentinal tubules and presence of smear-layer*,* bonding to dentin still lacks improvements that guarantee the durability of the adhesive interface (5, 19).

Smear layer, a structure formed by the debris resultant from the cutting process, may present different composition, thickness and morphology, depending on the location and type of burs used (8). Hence, this structure can obliterate the dentinal tubules entrance, reducing their permeability to the penetration of the adhesive system and therefore making the adhesion to dentin substrate dependent on the type of smear layer pre-treatment (3, 21). The main strategies to achieve effective dentin adhesion are: etch-and-rinse protocol which requires the conditioning of substrate with phosphoric acid, thus removing the smear layer; and self-etch mode which uses a non-rinse acidic primer, leading to a smear layer dissolution, with no demineralization of the subsurface and the promotion of resin infiltration (3, 5, 6). Moreover, self-etching primers are less sensitive to dentin variability than total-etch adhesives. The absence of acid conditioning allows the maintenance of mineral content, preventing unprotected dentinal collagen fibrils which are highly vulnerable to enzymatic and hydrolytic degradation, consequently promoting a longer durability of adhesive interface (5, 8).

Conventionally, dentin adhesion is guaranteed by the formation of resin tags inside the dentinal tubules, allowing the mechanical interlocking of the hybrid zone (1, 8). Currently, it is known that this bond results from three phenomena: mechanical interlocking, surface bonding and, primarily, collagen network (1). In light of these evidences, the quality of intertubular dentin might be the key for successful dentin bonding, thus it should be treated properly in order to be preserved (1, 4).

When aiming to evaluate and characterize dentin adhesion, several strategies may be used. In this study, microtensile bond strength tests were used to evaluate bond strength to dentin of three adhesive systems after sandblasting with Al_2O_3 as a dentin pre-treatment. Microtensile bond strength is calculated as the maximum tensile load at failure divided by the cross-sectional area of the adhesive interface (23). The evaluation of adhesion strength of restorative materials through *in vitro* studies allows to foresee their clinical behaviour and success in short and long-term (5). Currently no consensus exists

in dentistry for μTBS tests, making the comparison across studies difficult, since variable parameters and methods have been used (23). Although there are no broad agreements and standardized approaches of testing methodology, these studies present several advantages such as simplicity and speed, testing large number of samples and measurement of just one experimental parameter, thus being more versatile than conventional tensile or shear methods (5, 23).

In order to obtain a uniform and standardized smear layer, sandpapers of distinct grit sizes were used in this study. This technique provides a flat dentin surface with fewer irregularities and the standardized smear layer generated is indicated to be used with different surface treatments, thus allowing their comparison (5).

Adhesive failures are the only ones that should be considered for the μTBS calculation, so that accurate measurements are achieved (5). However, since this is a pilot study and a small number of specimens was tested, all test results were considered, including cohesive failures, which might not reflect true bond strength. Moreover, a minimum of 30 specimens should be accessible for testing in order to attain reliable bond strength data (5).

Air abrasion, classified as a surface mechanical treatment, generates a roughened dentin surface, increasing the contact area for adhesion and thus improving the interfacial contact between substrate and adhesive (1, 3, 5, 19). According to Rafael *et al.*, Al_2O_3 air abrasion as a dentin pre-treatment allows the preservation of intertubular dentin, maintaining the original diameter of dentinal tubules entrances (1).

This technique, depending not only on the operator's experience, might be influenced by several factors such as tip diameter, air pressure, distance to dentin surface, application time, particle size and tip angulation (18). Additionally, Chayiabutr and Kois, stated that, despite particle size does not directly influence adhesion strength, smaller particles create a more retentive pattern and may lead to a stronger adhesive interface (14).

The results of this study showed that sandblasting with aluminum oxide particles as a dentin cleansing method decreased the bond strength for all adhesive systems tested, thus the first null hypothesis was rejected. Nevertheless, no interaction was found between the type of the adhesive system and Al_2O_3 air abrasion, as microtensile bond strength did not differ among adhesives, therefore the second null hypothesis was accepted. Although some other authors stated that this method does not negatively influence dentin bond strength (4, 5, 8, 21), in the present study air abrasion seems to have a deleterious effect in adhesion to dentin.

Soares *et al*. noted that μTBS of one-step self-etch adhesive system was not influenced when dentin was air abraded with Al_2O_3, with bond strength values around 25.2MPa (4). Anja et al. observed that the use of air abrasion with the same category of self-etch adhesive does not enhance μTBS in dentin, obtaining values around 35.8MPa (5). Similarly, Chaves *et al*. observed that when phosphoric acid etching was substituted for Al_2O_3 air abrasion, no improvement in dentin bond strength was noted for etch-and-rinse adhesives, with μTBS of 38.4MPa and 38.6MPa, respectively. Also, bond strength did not significantly increase when self-etch adhesives were used (8).

Previous studies, using sandblasting as a dentin surface treatment, reported that the surface roughness promoted by the impact of the Al_2O_3 particles and the increase of the contact area could be beneficial for adhesion, since the mechanical retention would be enhanced (18, 19, 24). Regardless the fact that the surface roughness obtained with dentin sandblasting did not enhance the bond strength in the present study, is possible to infer that this characteristic is not the only factor to influence adhesion. According to Anja *et al.*, physical parameters and the chemical composition of dentin substrate also influence adhesion (5).

Additionally, in SEM observations, comparing groups 1 and 4, the self-etch adhesive is apparently unable to dissolve the smear-layer produced by Al_2O_3 abrasion, hampering the resin monomers infiltration. Likewise, in group 2, the dense smear layer produced by dentin sandblasting might act as an obstacle to adhesion, considering that acid etching was not effective in its removal. In agreement with these results, in a previous pilot study, Cruz *et al*. described the creation of a dense and amorphous smear layer after dentin sandblasting, altering the interaction pattern between the adhesive systems and the substrate (25). In addition, Chinelatti *et al*. stated that dentin treated with Al_2O_3 presents lower infiltration of the adhesive system, and the combination with acid conditioning provided the formation of a few resin tags mainly due to the reduction of the Al_2O_3-created smear layer (19). On the contrary, in group 4, where dentin was not sandblasted, the hybrid layer is more homogeneous and continuous. Also, in group 5, the adhesive interface is well-defined, presenting resin tags and opened dentinal tubules with funnel shape, accordingly to an adequate adhesive pattern (19).

In SEM analysis of groups 3, it is possible to observe the scarce existence of resin tags, thus being possible to infer that the permeability of the dentinal tubules is significantly reduced, since the universal adhesive system, when applied in self-etch mode, appears to be unable to sufficiently dissolve the smear layer. In group 6 the hybrid layer is more homogeneous, presenting a greater continuity between the adhesive and the dentin

substrate, as well as a deeper infiltration of the resin monomers in the dentin tubules. Regarding group 3, the lower number of resin tags might be due to the pH of the Scotchbond™ Universal, which, although classified as a mild adhesive system, has a higher pH value of 2.7, resulting in a less acidic composition and, consequently, with a lower efficiency in dissolving the dense smear layer created by dentin sandblasting (2).

In the study of Burnett *et al*., results showed that there was no statistically significant difference in μTBS of a universal adhesive applied in etch-and-rinse mode when Al_2O_3 was used (21).

For ScotchBond™ Universal, Sutil *et al*. noted that, when the adhesive was used in the etch-and-rinse mode, μTBS increased significantly when the dentin was abraded with Al_2O_3, obtaining a mean value of 44.26MPa, while control group obtained values around 30.10MPa (3). However, when ScotchBond™ Universal was used in self-etch mode, air abrasion did not increased dentin bond strength, achieving a mean value of 37.46MPa, while control group obtained results around 36.14MPa (3). These findings support the statements of Atoui *et al*., that argue that the roughened surface created by air abrasion restricts the penetration of the adhesive monomer when the modified dentin surface is not etched with phosphoric acid, compromising the adhesive layer durability (6). Some authors advise to perform dentin etching after Al_2O_3 air abrasion, not only to remove the so-called dense smear layer, providing higher permeability, but also to remove Al_2O_3 debris left on the dentin surface, since these particles may influence the resin monomer infiltration (3, 19, 21). Notwithstanding, comparing groups 2 and 5, it was possible to observe that, even after phosphoric acid etching of sandblasted dentin, there might be a worse adhesion quality, since acid conditioning seems unable to remove the smear-layer resultant of Al_2O_3 abrasion, leading to lower values of μTBS.

In clinical practice, Al_2O_3 air abrasion requires some additional precautions. Besides the fact that isolation of the working field is crucial, an adequate suction of aluminum powder cloud is mandatory to avoid inhalation of Al_2O_3 particles during the procedure (4, 22). Moreover, difficulties related to the sandblasting standardization, such as maintaining pressure, angulation and distance, and ensuring that the procedure duration does not exceed the recommended time, represent a significant clinical challenge (14).

Conclusions

Based on the findings of the literature and within the limitations of this pilot study, it was found that:

1. Sandblasting decreased microtensile bond strength to dentin;
2. No interaction was found between adhesive systems and sandblasting with $Al_2O_{3;}$
3. Micromorphology revealed a high-level resin tag penetration and anastomosis when dentin was not pre-treated with $Al_2O_{3.}$

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Table of Contents