©Operative Dentistry, 2022, **47-1**, 87-96

Effect of Time and Temperature of Air Jet on the Mechanical and Biological Behavior of a Universal Adhesive System

R Zimmer • ML Leite • CA de Souza Costa • J Hebling
G Anovazzi • CA Klein-Junior • K Hosaka • ED Reston

Clinical Relevance

Insufficient polymerization of resinous materials increases the toxicity of these products and results in the formation of a more fragile polymer. The application of hot air jet blast favors the polymerization of the material, reducing its cytotoxicity and increasing its adhesion to dentin.

SUMMARY

Objectives: To evaluate the influence of heat application on the degree of conversion (DC) of the 3M Single Bond Universal Adhesive System, as well as its transdentinal cytotoxicity and microtensile bond strength to dentin.

Methods: Experimental groups were established according to the time and temperature of the air jet: G1: 5 seconds–25°C; G2: 10 seconds–25°C; G3: 20 seconds–25°C; G4: 5 seconds–50°C; G5: 10 seconds–50°C; G6: 20 seconds–50°C. In control group (G7), no treatment was performed. The DC

Roberto Zimmer, DDS, MS, Department of Operative Dentistry, School of Dentistry, Lutheran University of Brazil (Ulbra), Canoas, Brazil

Maria Luisa Leite, DDS, MS, PhD, Department of Physiology and Pathology, Araraquara School of Dentistry, São Paulo State University (Unesp), Araraquara, Brazil

Carlos Alberto de Souza Costa, DDS, MS, PhD, Department of Physiology and Pathology, Araraquara School of Dentistry, São Paulo State University (Unesp), Araraquara, Brazil

Josimeri Hebling, DDS, MS, PhD, Department of Orthodontics and Pediatric Dentistry, Araraquara School of Dentistry, São Paulo State University (Unesp), Araraquara, Brazil

Giovana Anovazzi, DDS, MS, PhD, Department of Orthodontics and Pediatric Dentistry, Araraquara School of Dentistry, São Paulo State University (Unesp), Araraquara, Brazil

Celso Afonso Klein-Junior, DDS, MS, PhD, Department of Operative Dentistry, School of Dentistry, Lutheran University of Brazil (Ulbra), Canoas, Brazil

Keiichi Hosaka, DDS, MS, PhD, Department of Cariology and Operative Dentistry,Graduate School of Medical and Dental Sciences,Tokyo Medical and Dental University, Tokyo, Japan

*Eduardo Galia Reston, DDS, MSD, PhD, Department of Operative Dentistry, School of Dentistry, Lutheran University of Brazil (Ulbra), Canoas, Brazil

*Corresponding author: Predio 59, 3o Andar Av Farroupilha, 8001- São José, Canoas - Rio Grande do Sul – Brasil 92425-020; e-mail: ereston@dentalcore.com.br

http://doi.org/10.2341/20-038-L

was assessed using the Fourier transform infrared spectroscopy–attenuated total reflectance (FTIR–ATR) technique. For the transdentinal cytotoxicity test, dentin discs fitted in artificial pulp chambers (APC) received the application of the adhesive system and the air jets. For the microtensile bond strength, healthy molars were restored and submitted to the microtensile test after 24 hours and 6 months, respectively.

Results: Significant reduction in viability of Mouse Dental Papilla Cell-23 (MDPC-23), which exhibited morphological changes, was observed in all experimental groups compared to control ($p<0.05$). Although all tested protocols resulted in transdentinal diffusion of 2-hydroxyethyl methacrylate (HEMA), the group G6 presented the highest degree of monomeric conversion and the lowest cytotoxic effect, with higher dentin bond strength values in comparison to group G1 ($p<0.05$).

Conclusions: Applying an air blast at 50°C for 20 seconds increases the DC and microtensile bond strength of the 3M Single Bond Universal Adhesive System to dentin, as well as reduces the transdentinal cytotoxicity of the material to pulp cells.

INTRODUCTION

Universal adhesive systems were developed with the objective of facilitating the adhesive restoration technique. In order to concentrate the multipurpose characteristics of this material into a single vial, a high solvent content was added to new monomeric components, which may diffuse through dentin to reach the pulp tissue.[1-7] Uncured resin monomers in contact with the dental pulp trigger persistent chronic foreign body inflammatory reactions and cause irreversible toxic effects to pulp cells.[8-11]

It is known that during the curing of adhesive systems, there is an incomplete conversion of monomers that remain free in the dentin[12] and that, due to substrate permeability, can diffuse via the dentinal tubules to cause pulp damage.[13,14] Thus, it is essential that the polymerization process is effective, maximizing the physical-mechanical properties, favoring the clinical performance of the product, as well as reducing its possible toxic effect on dental pulp cells.[7]

Due to the fact that the cytotoxicity of resinous materials is directly related to the effectiveness of the polymerization technique,[2,7] some protocols have been tested to increase the degree of conversion (DC) of monomers to polymers. Rising temperature facilitates solvent evaporation, activates free radical mobility, reduces viscosity, and makes polymer chains more flexible, increasing the extent to which monomers can be converted into polymers.[15-20] Thus, by evaluating different heat-curing polymerization methods, some authors have reported improved physical, mechanical, and biological properties of resinous materials.[17,20-28]

As a result, the application of a heat source to the adhesive system may enhance the conversion of monomers to polymers, increasing mechanical properties and reducing material cytotoxicity. Thus, the aim of the present study was to evaluate the influence of heat application on the DC, transdentinal cytotoxicity, and dentin bond strength of a universal adhesive system.

METHODS AND MATERIALS

To perform the experimental tests, the 3M Single Bond Universal Adhesive System was selected (3M Oral Care, 3M Deutschland, Seefeld, Germany; Table 1), based on its previous performance in several studies over time. For the application of the air jet blast, a thermal blower (GuangZhou YiHua Electronic Equipment Co. Ltd., China) was used, modified to promote an air flow at different temperatures, which can vary from room temperature to 70°C, in addition to the use of a tip compatible with the size of a dental cavity.

Degree of Conversion

The degree of conversion (DC) was assessed by a spectrometer (VERTEX 70v, Bruker Optics, Ettlingen, Germany) using the Fourier Transform Infrared Spectroscopy (FTIR) technique equipped with an attenuated full reflectance accessory (ATR)—FTIR–ATR. Aliquots of 5 μL of the adhesive system were applied directly over the diamond crystal to read the material spectrum.[28]

Unpolymerized material was analyzed, followed by specimen preparation (n=3). The specimens were air jet blasted with varying times and temperatures, as shown in Table 2, and photoactivated (VALO, Ultradent Products Inc, Salt Lake City, Utah, USA) for 10 seconds. The percentage of unreacted carbon–carbon double bonds was determined by the ratio of the absorbance intensities between the aliphatic (peak at 1636/cm) and aromatic carbon–carbon (peak at 1608/cm) double bonds at a resolution of 4/cm and 32 scans.

Transdentinal Cytotoxicity

A total of 106 healthy human molars were obtained from the Tooth Bank of the Lutheran University of Brazil, after ethical approval. Using a metallographic

Table 1: *Composition of the 3M Single Bond Universal Adhesive System (3M Oral Care)*	
Adhesive System	**Composition**
Single Bond Universal	Bisphenol Diglycidyl ether dimethacrylate (BisGMA), 2-hydroxyethyl methacrylate (HEMA), silica treated silica ethyl alcohol, decamethylene dimethacrylate, water, 1-10 decanediol phosphate methacrylate, acrylic and itaconic acid copolymer, camphorquinone, N, N -dimethylbenzocaine, 2-dimethylaminoethyl methacrylate, methyl ethyl ketone

cutter (IsoMet 1000, Buehler Ltd, Lake Bluff, IL, USA) equipped with a diamond blade (11-4254, 4"x0.012"/15LC series, Diamond Wafering blade, Buehler Ltda), 0.4 mm dentin disks were obtained from 70 teeth. These discs had their hydraulic permeability determined as previously described by Leite and others.[5] The diameter of the discs was reduced to 8 mm with a high speed cylindrical diamond tip (Diamond Tip FG 3098—KG Sorensen). The discs were then adapted in artificial pulp chambers (APC), and the disc–APC sets were subjected to ethylene oxide sterilization.[5]

Immortalized cells of Mouse Dental Papilla Cell-23 (MDPC-23) odontoblastic lineage stored in liquid nitrogen at the Laboratory of Experimental Pathology and Biomaterials at the Dental School-São Paulo State University (FOAr/UNESP) were thawed and cultured in 100 cm^2 plates (Costar Corp, Cambridge, MA, USA) in Dulbeccos's Modified Eagle Medium culture medium (DMEM; GIBCO, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS, Cultilab, Campinas, SP, Brazil), 100 IU/mL and 100 μg/mL of penicillin and streptomycin, respectively (GIBCO, Grand Island, NY, USA). These cells were subcultured and kept in an incubator containing 5% CO_2 at 37°C until they reached sufficient number to perform the experiment.

Sterile disc–APC assemblies were individually inserted into 24-compartment plates so that the pulp surface of the dentin discs faced upwards. On this surface, 1×10^5 cells were seeded in 20 μL of complete DMEM. After 30 minutes in an incubator (sufficient time for initial adhesion of cells to the dentin substrate), 1 mL of complete DMEM was applied to each compartment of the plates, which were kept in an incubator for an additional 48 hours. At the end of this period, the culture medium was replaced with 1 mL FBS-free DMEM and the disc–APC assemblies inverted in the compartments such that cells adhered to the pulp surface of the disc were kept down and in contact with the DMEM. Thus, the occlusal surface of the dentin discs, now facing upwards, remained exposed to receive the treatments proposed in the present study (Table 2).

Table 2: *Relationship Between Groups and Protocols of Air Jet Application*	
Groups	**Protocols**
G1 [a]	5 seconds of air jet at 25°C
G2	10 seconds of air jet at 25°C
G3	20 seconds of air jet at 25°C
G4	5 seconds of air jet at 50°C
G5	10 seconds of air jet at 50°C
G6	20 seconds of air jet at 50°C
[a]*Protocol recommended by the manufacturer.*	

To perform the adhesive protocols, the occlusal surface of each dentin disc was washed with 1 mL of Phosphate-buffered saline (PBS) with concomitant aspiration, and the excess moisture was removed with sterile absorbent paper. Next, the total volume of 10 μL of the adhesive system was applied for 20 seconds over all dentin-exposed surfaces followed by gentle air jet blast application (Table 2) and photoactivated for 10 seconds with high power LED (VALO, Ultradent), with a light intensity of 1000 mW/cm^2. For this cytotoxicity test, a negative control group (G7) was established where no treatment was performed on the occlusal surface of the dentin discs. Next, the disc–APC sets were incubated in a 5% CO_2 atmosphere at 37°C for 24 hours.

To perform the cell viability test (n=8), the dentin discs were carefully removed from the APCs and individually positioned with the pulp surface containing the cells facing upwards at the bottom of the compartments of new 24-compartment acrylic plates. Next, the culture medium was aspirated with 90 μl DMEM and 10 μl MTT solution (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) (Sigma Chemical Company, St Louis, MO, USA) at a concentration of 5 mg/mL and was applied to each dentin disc. The samples remained for 4 hours in an incubator for formazan crystal formation. A solution with 100 μL of acidified isopropanol was then applied to the discs to dissolve these crystals. After dissolution, 100 μL aliquots were transferred to a 96-well plate (Costar Cat 3595—

Corning Inc, NY, USA) and the medium blue violet stain was quantified on a spectrometer (Synergy H1, BioTek, Winooski, USA) at a wavelength of 570 nm.

To evaluate the cell morphology, dentin discs (n=2) were removed from the APCs, and cells adhered to their surface were fixed with 2.5% glutaraldehyde, washed with PBS, and post-fixed with osmium tetroxide.[5] After being dehydrated in growing ethanol solutions (30%, 50%, 70%, 95%, and 100%), the specimens were chemically dried in 1,1,1,3,3,3- hexamethyldisilazane solution (HMDS, ACROS Organics, New Jersey, USA), mounted on metal stubs, and kept in a desiccator for 72 hours. The pulp surface of the dentin discs containing the cells was covered with gold and analyzed by scanning electron microscopy (JSM-6610; JEOL Ltd, Akishima, Tokyo, Japan).

To quantify the 2-hydroxyethyl methacrylate (HEMA) (n=6), 200 μL aliquots of the extracts (culture medium + dentin-diffused adhesive system components) representative of each group were collected and immediately applied to 96-well plate compartments with specific UV treatment (Corning Costar, New York, USA), and analyzed at an absorbance peak of 231 nm. HEMA concentration was determined by comparative method using a 6-point standard curve with 1:1 serial dilution (10 mM, 5 mM, 2.5 mM, 1.25 mM, 0.625 mM, and 0.312 mM), adapted by a previous study.[28] The mean of the negative control group was used as a blank.

Microtensile Bond Strength

Of the 106 healthy molars selected for the present study, 36 were transversely cut in the occlusal-third of the crown with the aid of a metallographic cutter (IsoMet 1000, Buehler Ltd) equipped with a diamond disk. Next, the sectioned surfaces of the teeth were worn with 600-grit sandpaper mounted on Politriz (ERIOS-27000, Euros, São Paulo, SP, Brazil). This procedure was performed under constant water cooling until a regular and homogeneous dentin surface was obtained, which was inspected with the aid of a stereoscopic magnifying glass (Model SZX7, Olympus, São Paulo, SP, Brazil).

To provide a homogeneous smear layer, the previously cut teeth had their dentin surfaces sanded for 30 seconds with 320-grit sandpaper (T469-Norton, Saint-Gobain Abrasivos Ltda, Jundiaí SP, Brazil). The dentin surface was then washed with distilled water, and the excess moisture was removed with absorbent paper, leaving the surface slightly damp. Next, 20 μL of the adhesive system was applied to the exposed dentin surface. After 20 seconds, solvent evaporation and material photoactivation were performed as described above. The teeth were restored with Filtek Z350 XT composite resin, color A 3.5 (3M from Brazil Ltda, Sumaré, SP, Brazil) using an incremental technique (three increments of 1 mm each, photoactivated for 20 seconds). After restoration, the teeth were stored in deionized water at 37°C for 24 hours.

With the aid of a metallographic cutter (IsoMet 1000, Buehler) equipped with a diamond disc, it was possible to obtain a total of eight sticks from each tooth (0.9×0.9 mm cross-sectional area). Half of the sticks were submitted to the microtensile bond strength test within 24 hours after the restorative procedure. The remaining sticks were immersed in saliva solution (KCl 12.92 mM, KSCN 1.95 mM, $Na_2SO_4 \bullet 10H_2O$ 2.37 mM, HEPES 5 mM, NH_4Cl 3.33 mM, $CaCl_2 \bullet 2H_2O$ 1.55 mM, $NaHCO_3$ 7.51 mM, $ZnCl_2$ 0.02 mM, pH 7.4) and then stored in an incubator at 37°C for 6 months, where they were submitted to the same mechanical test. During the storage period, the saliva solution was renewed monthly.

The specimens were fixed in metal devices of a universal mechanical testing machine (DL 1000, EMIC Testing Systems, São José dos Pinhais, PR, Brazil) using a cyanocrylate adhesive (Super Bonder Gel and Activator 7456, Henkel Loctite Ltda, São Paulo, SP, Brazil). Traction movements were initiated by a specific computer program (Test Works, Star IV, MTS System Corporation, Eden Prairie, MN, USA) and were terminated at the time the specimen ruptured with maximum load values recorded by the program. The fractured specimens were analyzed with a stereoscopic magnifying glass (Model SZX7, Olympus) having a 30-fold increase, and fractures were classified as resin or dentin cohesive, adhesive, or mixed.[30]

Statistical Analysis

Data on the DC, bond strength, and quantification of HEMA diffusion were evaluated by the two-way ANOVA test, followed by the Tukey test for DC and microtensile bond strength. For each period of analysis, the mean of the values of four sticks of each tooth was considered as an experimental unit (n=6). For cell viability, the results were submitted to the one-way ANOVA test, followed by the Tukey test for cell viability. All statistical tests were considered at a significance level of 5%.

RESULTS

Degree of Conversion

The highest monomeric conversion values were observed in the group where the adhesive system was heated by applying a 50°C air jet for 20 seconds, as shown in Table 3.

Table 3: *Average of the Degree of Conversion (DC) Values (%) According to Air Jet Temperature and Time*[a]

Time	Temperature	
	25°C	50°C
5s	55.8 ± 1.7 Aa (G1)	53.4 ± 1.5 Ca (G4)
10s	56.7 ± 0.7 Aa (G2)	60.4 ± 1.9 Bb (G5)
20s	57.3 ± 1.6 Aa (G3)	66.4 ± 3.1 Ab (G6)

[a]*Lowercase letters indicate difference between temperatures (same line); uppercase letters indicate time difference (same column).*

Transdentinal Cytotoxicity

Cell Viability—

For all groups, the adhesive systems caused a high cytotoxicity index when compared to the negative control group ($p<0.05$), as observed in Figure 1. However, in the group where the adhesive system received application of 50°C air jet blast for 20 seconds, the cells showed significantly higher viability than those belonging to the group where the material was blown at 25°C for 5 seconds ($p<0.05$).

Cell Morphology—

As shown in Figure 2, MDPC-23 pulp cytotoxicity occurred in all experimental groups, when compared to the negative control. This toxic action resulting from the treatments was determined by the reduction in the number of cells that remained attached to the pulp surface of the dentin discs, as well as by the morphological alteration of these cells, which had reduced size associated or not with the rupture of the cytoplasmic membrane. The most intense negative effects on cells were observed in group G1, where the adhesive system was blown at 25°C for 5 seconds.

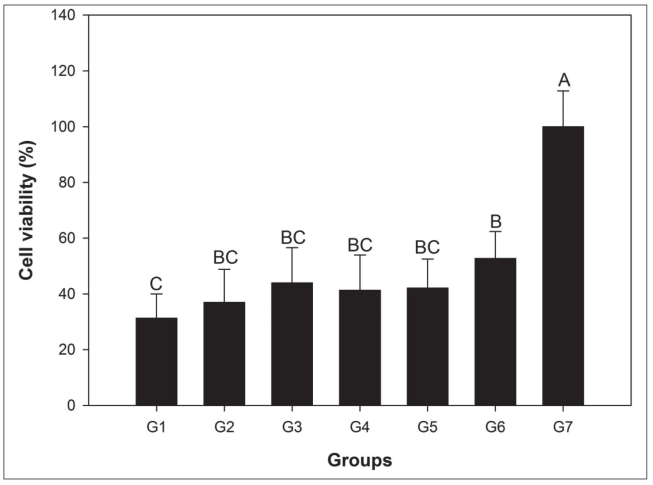

Figure 1. *Mean and standard deviation of cell viability values (%) of experimental and control groups. Different letters show significant difference between groups (*p*<0.05).*

HEMA Diffusion Quantification—

Although the amount of HEMA diffused through the dentin was not statistically different when the experimental groups were compared with each other ($p>0.05$), it was possible to observe a tendency of reduction in HEMA diffusion values with increasing the time and air jet temperature applied over the adhesive system (Figure 3).

Microtensile Bond Strength

The failure mode distribution of the specimens in both the periods of analysis is shown in Table 4. In general, it was observed that the failures occurred predominantly at the adhesive interface.

As shown in Figure 4, the increased air jet application time resulted in an increase in dentin bond strength, both in the 24-hour and 6-month analyses. Similarly, the use of heat over the same period of time resulted in better dentin bond strength results. The increase in air jet time and temperature provided an increase in bond strength.

It is noteworthy that the application protocol of the dentin adhesive system recommended by the manufacturer presented the least favorable results of bond strength in the 24-hour analysis, and which were even worse in the 6-month period. Differently, the application of an air jet at 50°C for 20 seconds presented the best results in the analysis after 6 months.

DISCUSSION

Increasing temperature provides greater mobility of the photoinitiators and monomers present in the resin matrix, as well as an increase in molecular kinetic energy, facilitating breakage of intermolecular bonds between solvents and resin monomers.[17,31] Consequently, the combination of higher solvent evaporation and increased vibrational energy of molecules favors

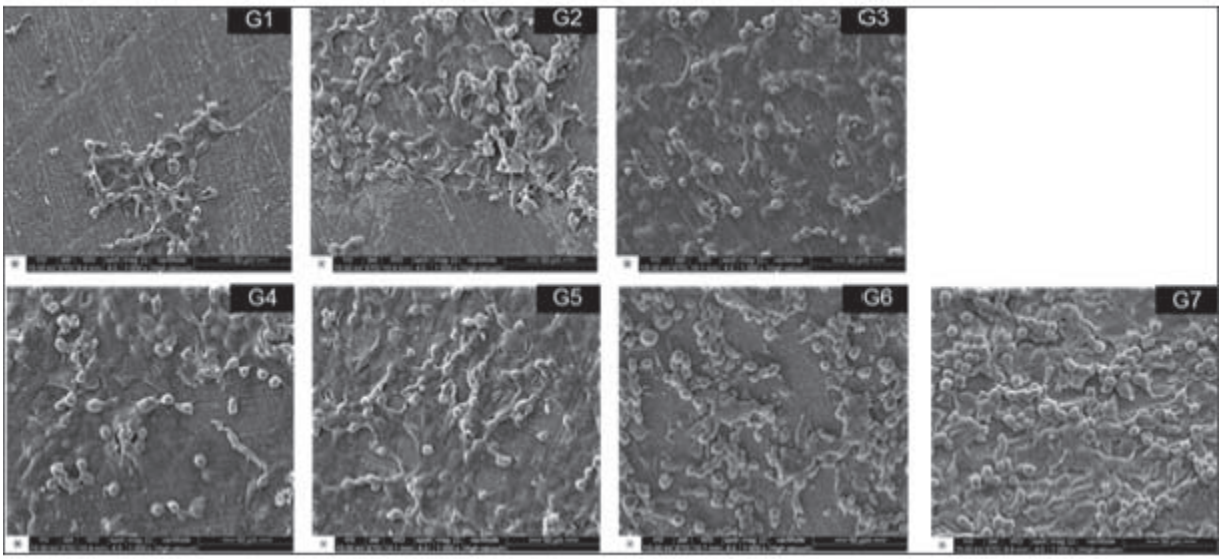

Figure 2. *SEM, 1000× Representative images of Mouse Dental Papilla Cell-23 (MDPC-23) cells sown on the surface and pulp of the dentin discs of the experimental and control groups. G1–G6: The application of the adhesive system on the occlusal surface of the dentin discs caused intense cytotoxicity response into the MDPC-23 odontoblast cells shown on the pulp surface, promoting morphological changes such as size reduction and loss of cytoplasmic processes. The most intense degree of cytotoxicity was presented in G1, where a large number of damaged cells detached from the dentin disc. G7 (control): MDPC-23 cells displaying an intact cytoplasmic membrane covering virtually all dentinal substrate.*

monomeric conversion,[20] reducing the amount of free monomers with potential to diffuse through the dentin to cause damage to the pulp cells.[1-3,5]

In the present study, it was possible to identify a higher degree of monomeric conversion by increasing the time and temperature of the air jet applied over the adhesive system. Previous studies used temperatures from 37°C to 60°C to promote greater solvent evaporation and increase the DC.[20-22,31-35] Silva and others[36] demonstrated that applying 60°C air jet blast on 0.5 mm thick dentin for 10, 20, 30, and 40 seconds increased the pulp temperature by 5.8°C, 10.1°C, 13.6°C, and 16.6°C, respectively. These thermal variations may possibly cause pulp damage, since it has been reported that an increase between 5.5°C and 11°C in the intrapulp temperature results in different levels of primate pulp necrosis.[37] Under other conditions, Baldissara and others[38] demonstrated that a 9-15°C increase in intrapulp temperature was not sufficient to cause pulp necrosis after 3 months.

In the present research, two temperatures (25°C and 50°C) and three application times of air jet for solvent

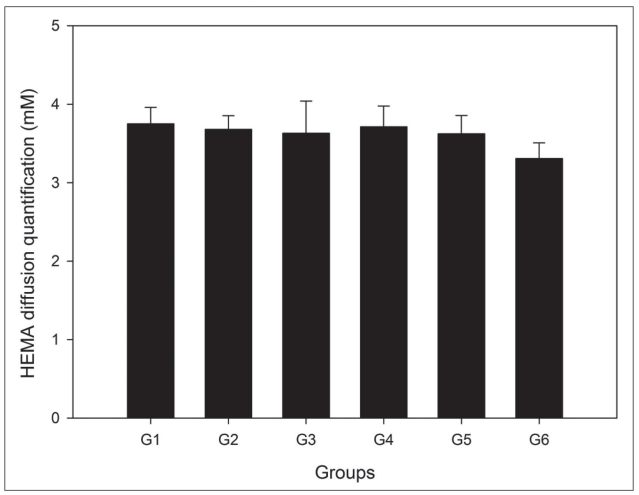

Figure 3. *Mean and standard deviation of 2-hydroxyethyl methacrylate (HEMA) diffusion quantification values (mM). There was no statistical difference between groups.*

Table 4: *Types of Fractures Presented During μTBS 24 h and 6 Months*

Groups	Types of Fractures							
	24 h				6 m			
	A	M	D	R	A	M	D	R
G1	19	1	3	1	15	2	3	4
G2	16	2	1	5	13	4	3	4
G3	14	2	1	7	15	0	7	2
G4	18	1	2	3	15	0	1	8
G5	16	0	6	2	13	3	3	5
G6	14	3	3	4	13	2	1	8

Abbreviations: A, adhesive; D, dentin cohesive; M, mixed; R, resin cohesive.

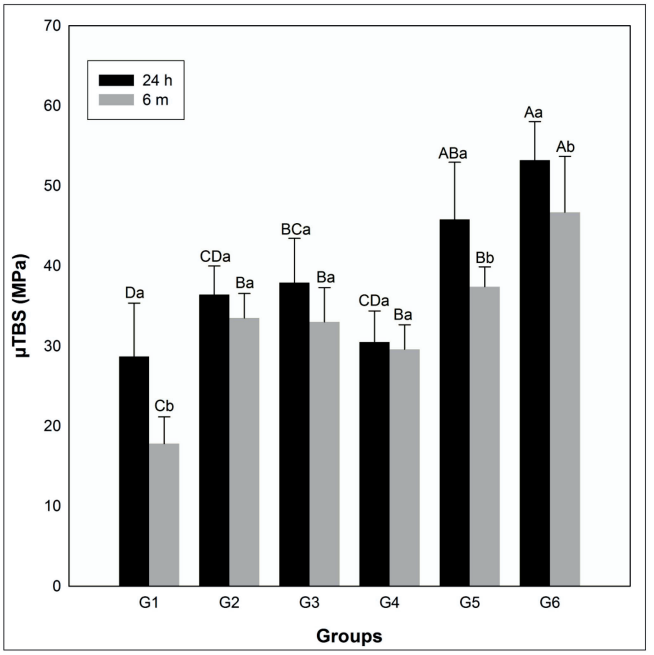

Figure 4. *Mean and standard deviation of the bond strength values in the immediate and late analyses (n=6; ANOVA test; α =0.05). Different upper case letters show significant difference between the groups in the same analysis period, while different lower case letters indicate significant difference of each group in the different analysis periods.*

evaporation of the adhesive system (5, 10, and 20 seconds) were evaluated. In all experimental groups, there was a significant reduction in cell viability compared to the control group (G7). Overall, the cell damage observed in the present study was not only limited to reduced mitochondrial activity but was also characterized by morphological changes in pulp cells, which exhibited cytoskeleton contraction and loss or shortening of cytoplasmic prolongation. In these experimental groups, a reduction in the number of cells adhered to the dentin substrate was also observed, indicating the occurrence of cell death. However, it was determined that the smallest damage occurred when a 50°C air jet blast was applied for 20 seconds over the occlusal surface of the dentin discs. Thus, in addition to increasing the degree of monomer conversion,[20] the air jet blast at a temperature of 50°C was the least aggressive to the pulp cells. This positive result regarding cell number maintenance and viability may have been due to the fact that fewer free monomers were able to diffuse through the dentin to cause toxic effects on the pulp cells. Previous studies have shown that transdentinal toxicity of adhesive systems is directly related to the amount of free monomers released from the material that can diffuse through the dentinal tubules.[1,39] Thus, higher cytotoxicity was observed in the group where the 25°C air jet blast was directed for 5 seconds (manufacturer's recommendation) on the adhesive system applied to the occlusal surface of the dentin disc. The scientific data obtained in the present research supports the idea that the higher the degree of monomer conversion the lower the cytotoxicity of resinous materials, as recently reported by Fujioka-Kobayashi and others.[40]

Although resinous materials have different types of monomers in their composition, which can cause toxic effects of varying intensity on pulp cells, HEMA is the major component of adhesive systems responsible for transdentinal toxicity.[6] Although no statistical difference was observed, there was a tendency for a lower transdentinal diffusion of HEMA with increasing time and temperature of the air jet applied over the adhesive system. Perduns and others[41] reported that slight variations in the amount of HEMA may influence the level of cytotoxicity of this resinous monomer on pulp cells. The authors demonstrated that 0.5 mM HEMA is sufficient to induce the expression of genes related to cellular oxidative stress; higher concentrations of this monomer result in overload to the antioxidant system, which causes cell death and modulates inflammatory and metabolic pathways that modify the extracellular matrix.

In a previous study, where the authors also used the 0.4 mm thick dentin discs model mounted on APCs, it was shown that the 3M Single Bond Universal Adhesive System, used in the conventional technique or as self-etching (wet or dry dentin), reduced cell viability by about 88%.[5] In the present study, by applying the same adhesive system as a self-etching agent on the wet dentin substrate, varying the time and temperature of the air jet for solvent evaporation, a reduction in cell viability was observed between 47.3% and 68.7%. It is possible to suggest that the higher transdentinal toxicity observed in the study by Leite and others[5] occurred due to the fact that the authors actively applied the adhesive system on the dentin, which did not happen in the present study. The active application technique is recommended by the manufacturer to favor the infiltration of resin monomers into demineralized dentin, which seems to result in the formation of a more homogeneous hybrid layer.[22] However, it is known that the active application of the adhesive on the dentin may also increase the transdentinal diffusion of free monomers to damage pulp cells.[42]

The use of standardized laboratory protocols, especially those employing standardized permeate dentin barriers to assess the indirect cytotoxicity of dental materials, is necessary to ensure the reliability and reproducibility of studies.[14,43] The use of dentin

barriers in *in vitro* research approximates laboratory protocols to clinical conditions and helps to understand how dentin characteristics may affect the diffusion of molecules from experimental materials at concentrations that may cause pulp cell toxicity.[1,14] Due to the need to assess the biocompatibility of dental materials and new clinical procedures, cell culture studies should be adopted as a prior approach to *in vivo* evaluations and clinical trials.[43] Even with all the advantages of *in vitro* studies, it is known that the results of these laboratory studies should not be directly extrapolated to clinical situations.[14] Thus, the authors of the present study recognize the limitation of the data obtained and the need for future-controlled clinical investigations, in order to determine the safe application of the procedures successfully tested here.

To evaluate the mechanical properties involved in the formation of the hybrid layer, the bond strength to dentin was evaluated. Klein-Junior and others[21] observed higher resin–dentin bond strength by applying an air jet blast at 60°C for 10 seconds, while Fu and others[44] and Saikaew and others[45] obtained better results by increasing the air jet application time. Rising temperatures are known to increase the kinetic energy of molecules, altering the way they bond together, favoring polymerization.[20] On the other hand, prolonging the air jet blast application time promotes greater solvent evaporation, resulting in a more efficient polymerization, which may favor the bond strength of the material with the dental substrate.[44,45] In the present study, the times of 5, 10, and 20 seconds of application of the air jets were tested, which associated with the increase of the temperature generally resulting in an increase of bond strength. The use of air jet blast, as recommended by the manufacturer (5 seconds), caused a reduction in bond strength, which was lower than in the other groups within 6 months of analysis. However, the application of 20 seconds of 50°C air jet resulted in increased bond strength, which was superior to the other groups evaluated within 6 months.

Finally, given the limitations of the present *in vitro* study, it is possible to suggest that the increase in temperature and time of application of the air jet over the 3M Single Bond Universal Adhesive System favors the DC and bond strength of the material to dentin, as well as decreasing its transdentinal cytotoxicity. In the present laboratory study, we simulated a very deep cavity through the use of 0.4 mm thick dentin discs. This protocol aimed to expose the adhesive system and the solvent evaporation techniques under test to a maximum toxicity challenge. However, regardless of the techniques evaluated in the present study, it is known that the application of adhesive systems in very deep cavities is still contraindicated, since these resinous materials, used as self-etching agents or on acid-etched dentin, cause intense pulp toxicity.[9,10,14] Therefore, the use of lining agents with important mechanical and biological properties has been recommended prior to the application of the adhesive system in cavities whose walls are very close to the pulp.[46]

CONCLUSION

According to the methodology employed in the present *in vitro* study, it was concluded that the application of air jet blast at a temperature of 50°C for 20 seconds favored the monomeric conversion of the 3M Single Bond Universal Adhesive System, reducing the transdentinal toxicity of this adhesive on the pulp cells as well as increased bond strength of the material to dentin.

Acknowledgments

The present study was partially supported by the Coordination for the Improvement of Higher Education Personnel - CAPES (Finance Code 001) and National Council for Scientific and Technological Development – CNPq (Grant # 408721/2018-9). The authors thank the "Banco de Dentes da Universidade Luterana do Brazil, Campus Torres," for providing all teeth used in the present investigation.

Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and policies of Lutheran University of Brazil. The approval code issued for this study is 89363218.3.0000.5349.

Conflict of Interest

The authors have no financial interest in any of the companies or products mentioned in this article.

(Accepted 5 March 2021)

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