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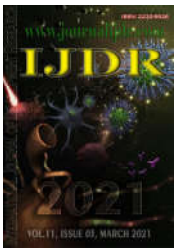

RESEARCH ARTICLE OPEN ACCESS

EFFECT OF NON-THERMAL ATMOSPHERIC PLASMA, ACID ETCHING, AND AGING ON THE BOND STRENGTH OF A UNIVERSAL ADHESIVE TO DENTAL ENAMEL

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ABSTRACT

This study in vitro evaluated the effect of non-thermal atmospheric plasma on the bond strength (BS) of a universal adhesive to human enamel, with or without acid etching when subjected to aging. Forty proximal enamel surfaces were randomly assigned to 4 groups according to acid etching (37% phosphoric acid) and plasma treatment (applied for 20 seconds by scanning mode). Afterwards, Scothbond Universal Adhesive was applied, and a composite resin block was built up. After 24 hours of storage, the specimens were sectioned into sticks. Half of the sticks in each group were immediately submitted to the μTBS test and the remaining after 12 months of aging. Data were submitted to three-way ANOVA with subdivided plots ($p < 0.01$) followed by Tukey test (<0.05). The three isolated factors showed statistical difference: time ($p < 0.001$), plasma ($p = 0.04$) and acid etching ($p = 0.006$). There was significant interaction only for time-acid etching ($p = 0.013$). The acid conditioning of enamel increased the BS values after immediate traction, effect not observed for plasma. Aging after 12 months significantly reduced the BS of all groups without statistical difference between them. The authors concluded that unlike phosphoric acid, plasma application did not increase enamel BS.

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INTRODUCTION

Despite the immediate and short-term bonding efficacy of dental adhesives, their durability and stability (De Munck*et al*., 2005; Inoue *et al*., 2001) remain under investigation, particularly when multimode universal adhesive systems (UA) (De Munck *et al*., 2005; Hanabusa *et al*., 2012) is used by the self-etch adhesion strategy (Cuevas-Suáres *et al*., 2019). Indeed, the weak action of Scotchbond Universal Adhesive (SBU) monomer on enamel compromised the in vitro long-termbonding between them (Vicenzi *et al*., 2019). As known, changes compatibility with the adhesive (Dong *et al*., 2015).In this field, the non-thermal atmospheric plasma (NTAP) gas (Cheruthazhekatt *et al*., 2010; D'Agostino *et al*., 2005) has been used to modify enamel/dentin surfaces (Liu *et al*., 2016; Han *et al*., 2014;Chen *et al*., 2013; Lehmann *et al*., 2013) in restorative proceduresto improve the adhesion, as well for other purposes (Ayres *et al*., 2018a; Abreu *et al*., 2016; Han *et al*., 2014; Ritts *et al*., 2010).Plasma (found naturally in the sun, stars, auroras, and rays) contains many highly reactive species, including electronically excited ions, electrons, free radicals, and neutrals (Awad *et al*., 2021; Chen *et al*., 2013).

It is generated when a gas becomes ionized (Hirata *et al*., 2015) and is composed of a low ionization degree of electrons (Tendero *et al*., 2006). Although a better wetting (by increasing active species, such as carboxylic groups) and surface free energy had facilitated the adhesive distribution when human dentin was NTAP pre-treated (Stasic *et al*., 2019), the knowledge about enamel is scarce to date (Almoammar *et al*., 2019; Teixeira *et al*., 2014; Lehmann *et al*., 2013). A recent study showed that NTAP increased the hydrophilicity of enamel and improved the marginal sealing of sealants by reduction the voids in the interface (Teixeira *et al*., 2014); this result suggests an advantage of applying NTAP when using a mild UA to make a composite resin restoration. Considering the selective etching of enamel margins with phosphoric acid (SEE) is still need when using SBU (Vicenzi *et al*., 2019) because the acidity of self-etching adhesives monomers (Cuevas-Suáres *et al*., 2019; Van Meerbeek *et al*., 2011),is possible that pre-treating enamel with NTAP would a) make SEE an unnecessary step, b) increase the bond strength even in long-term aging if the adhesive is applied by self-etch protocol, c) decrease the number of critical clinical steps (humidity control in acid etching and clinical time), and d) reduce the probability of failure. Based on the aforementioned, this in vitro study aimed to assess the effect of NTAP treatment on the bond strength of a universal adhesive system to human enamel, with or without enamel etching, when submitted to aging, simulating what occurs clinically with the restorations over time. The null hypotheses tested were NTAP, acid etching, and aging do not increase the bond strength of universal adhesives to human dental enamel.

MATERIALS AND METHODS

The present study was approved by Research Ethics Committee of the State University of Western Paraná (UNIOESTE) (CAAE 48197115.3.0000.0107). Twenty extracted unerupted human third molars were collected, cleaned, and stored in a 0.1% thymol solution under refrigeration before the experiment. Then, the roots were sectioned using a low-speed diamond saw.

Sample calculation: Sample size was calculated in terms of a minimum detectable difference in a mean BS (MPa) of 0.62 (30 human teeth, 8 per group), using the same adhesive system with and without enamel acid etching (Vicenzi *et al*., 2019).However, for predicting eventual losses, 10 samples were included.

Specimen preparation: The crowns were sectioned in the buccolingual direction using a 4"x0.012"x0.5" diamond cutting disc (EriosEquipamentosLtda - EPP, São Paulo SP, Brazil) attached to a precision saw (Labcut 1010, EriosEquipamentosLtda - EPP, São Paulo, SP, Brazil) at low speed to obtain two enamel proximal surfaces (samples 40 in total; 10 per group, 10 stick each, on average). First, the samples were flattened and polished with sequential silicon carbide abrasive papers up to the 2000 grit under water cooling, (Figure 1) and randomly assigned to two groups (n=20) according to the acid etching treatment (etched [Et], no-etched [no-Et]). For etching, 37% phosphoric acid gel (Condac 37, FGM, Joinville, SC, Brazil) was applied for 15 s, rinsed, and gently air-dried. Next, each group was subdivided into two (n=10) according to the NTAP treatment (plasma [P], no-plasma [no-P]) that consist of an argon unit as operating gas (Surface Plasma Tool Model, Surface Engineering and Plasma Solution, Campinas, SP, Brazil). The plasma torch (which emerged at the nozzle of 1 mm in diameter) was applied by scanning mode over the entire enamel surface for 20 s at room temperature of 22° C. The nozzle-enamel surface distance was kept in 10 mm (allowed by a movable base). Thus, the following groups were designed: Et/P, Et/no-P, no-Et/P, no-Et/no-P. Finally, the SBU was actively applied in all samples with a microbrush (20 s), air-dried (5 s), and light-cured (10 s) using a Valo Curing Light (Ultradent Products, INC., South Jordan, UT, USA) with an irradiance of 1000 mW/cm^2 and a composite resin block (Filtek Z100, 3M ESPE, St Paul, MN, USA) was incrementally built up (4 mm in thickness). The samples were kept for 24-hour in deionized water (37° C). Table 1 shows the compositions and manufactures of the materials.

Table 1. Composition and manufacturers of the materials used in this study

Materials, manufacturer	Composition
Scotchbond Universal Adhesive, 3M Oral Care, St Paul, MN, USA	Adhesive: 10-MDP, phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid, copolymer, filler, ethanol, water, initiators, silane
Z100, 3M Oral Care, St Paul, MN, USA	Silane treated ceramic, triethylene glycol dimethacrylate (TEGDMA), bisphenol A diglycidyl A ether dimethacrylate (BISGMA), 2-Benzotriazolyl-4-methylphenol
Condac 37, FGM, Joinville, SC, Brazil	37% phosphoric acid, thickener, pigment, deionized water

Microtensile bond strength (μTBS): After that, they were longitudinally sectioned in both 'x' and 'y' directions across the adhesive interface using a low-speed diamond saw attached to a precision cutting machine to obtain bonded sticks (1.0 mm±0.2 mm^2; 10 per sample) (Figure 1). Then, five sticks of each group were immediately [24h] submitted to the μTBS test and the other half after 12 months of aging [12m]. Sticks were attached to a testing jig with cyanoacrylate glue (Super Bonder Gel, Loctite, Henkel Ltda, São Paulo, SP, Brazil) and tested in tension in a universal testing machine (Emic-Instron, São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm/s.

The results of μTBS values were expressed in MPa. Statistical analysis was performed using the SigmaPlot 12.0 software at a 5% significance level, considering the plasma, etching, and aging as variation factors. The means of μTBS values among the specimens in each group were used for the statistical analysis. The variable passed the normality and homogeneity tests (Shapiro-Wilk/Kolmogorov-Smirnov) and was submitted to a three-way ANOVA with subdivided plots ($p<0.01$). Fractured surfaces were analyzed for failure mode by digital optical microscopy (Dino-Lite Premier, Anmo Electronics Corporation, New Taipei City, Taiwan) (magnification of 100%) and categorized according to the predominant remaining structure: adhesive in enamel (A); cohesive in enamel (CE); cohesive in resin (CR); or mixed (failure between adhesive/resin [M-AR] or among adhesive/resin/enamel [M-ARE]). The failure modes were expressed as percentages for each group.

RESULTS

The mean values of μTBS are shown in Table 2. The three isolated factors showed statistical difference: time (24h x 12m) ($p<0.001$), plasma (P x non-P) ($p=0.04$), and acid etching (Et x no-Et) ($p=0.006$). There was significant interaction only for the factor of time-acid etching ($p=0.013$). The enamel acid etching increased μTBSvalues when sticks were submitted to immediate traction testing, whereas plasma did not, even when combined with phosphoric acid. Overall, the highest μTBSvalue, which differed from all other groups, was observed for Et/no-P (52.7±13.8 MPa), followed by Et/P (40.9± 5.5 MPa), no-Et/no-P (37.2±16.9 MPa), and no-Et/P (32.9±11.8 MPa); the latter presented the lowest μTBS value. The 12-month aging reduced μTBSvalues for all groups, with no statistical difference among them. The μTBSranged from 20.3±3.8 MPa (for Et/no-P) to 17.68±15.1 MPa (for no-Et/P). Table 3 presents the failure rates of all groups.

DISCUSSION

The present study was planned to advance the understanding of the effect of NTAP, etching and aging on enamel adhesion by an established methodology (Ayres *et al*., 2018b; Liu *et al*., 2016; Cheruthazhekatt *et al*., 2010; Tendero *et al*., 2006).

Table 2. Microtensile bond strength means (MPa) obtained at 24 hours or after 12 months of aging

		Treatment			
		Et/no-P	no-Et/no-P	Et/P	no-Et/P
Evaluation Time	24 hours	52.7 (13.8)^{Aa}	37.2 (16.9)^{Bca}	40.9 (5.5)^{Ba}	32.9 (11.8)^{Ca}
	12 months	19.3 (5.3)^{Ab}	20.1 (3.8)^{Ab}	19.6 (3.8)^{Ab}	17.6 (15.1)^{Ab}

Mean (SD), n=20. Different superscript capital letters indicate significant difference among the means and compare treatments within the evaluation time. Different superscript lower-case letters indicate significant difference among the means and compare evaluation times within the same treatment. Three-way ANOVA ($p<0.01$). Plasma [P], non-plasma [no-P]), etched [Et], non-etched [no-Et], 24-hour aging [24h], 12-month aging [12m].

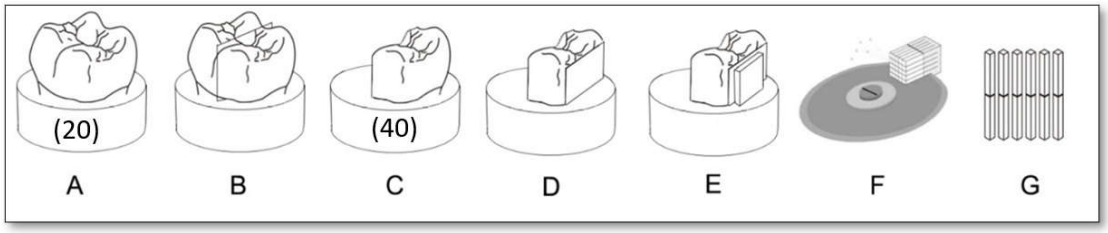

Figure 1. Schematic representation of the preparation of the specimens for the microtensile test. From a healthy tooth (A), sections were made in the VL direction (B, C). A flat enamel surface (D) was obtained, for which the restoration of composite resin was carried out according to the experimental groups (E). After serial cuts (F), toothpick specimens (G) were produced

It was expected plasma would increase surface wettability and consequently the bond strength between enamel and adhesive (Abreu *et al*., 2016; Han *et al*., 2014),as well acid etching. A direct comparison between the μTBS means values of Et and non-Et groups showed that etching increased μTBS significantly in samples tested immediately after 24-hour storage. Besides, those values were significantly reduced after 12 months of aging for all groups, illustrated by the percentage of A and M failures when the samples were not subjected to both treatments. The absence of CR failures for the no-Et/no-P groups indicates that there was a lack of ability of the adhesive to bind to the substrate, when applied without previous surface treatment. Thus, the null hypotheses of acid etching and aging would not affect the μTBS of universal adhesives to human dental enamel was rejected.

Table 3. Failure pattern incidence for the experimental groups

		Non-NTAP	NTAP
24 hours	Non-etched	A – 37%	A - 53,5%
		M – 63%	M – 46.5%
	Etched	A – 6,5%	A – 9.5%
		M – 56.5%	M – 65%
		Cr – 37%	Cr –25.5%
12 months	Non-etched	A – 49%	A – 64%
		M – 51%	M – 36%
	Etched	A – 35%	A – 12.5%
		M – 58%	M – 64%
		Cr – 7%	Cr –23.5%

Adhesive (A); Cohesive in resin (Cr); Mixed (M)

The present results are corroborated by other studies (Vermelho *et al*., 2017; Da Rosa *et al*., 2015; Goracci *et al*., 2013),except for the universal adhesive, which differ by trademark. According to studies (Da Rosa *et al*., 2015), adhesive system exhibited such a performance when evaluated in vitro. Indeed, a systematic review and meta-analysis (Cuevas-Suáres *et al*., 2019) showed a lower bond strength to enamel with self-etch adhesives than that of etch-and-rinse adhesives. So, dentists should etch the enamel margins with 37% phosphoric acid technique to achieve better clinical results when using universal adhesives. The way samples were affected by aging needs to be mentioned. Investigation of all dental adhesives categories showed mechanical and morphological evidence of degradation that resembles the effects of in vivo aging, after three months (De Munck *et al*., 2005).In the present study, samples tested after 12 months of aging in water indicated a significant reduction of μTBS, regardless of etching and NTAP treatments, as found others (Vermelho *et al*., 2017; Makishi *et al*., 2016). The high percentage of A/M failures for The no-Et/P group showed the positive effect of the etching procedure, considering that the μTBS values were high enough for the loss of composite resin rather than adhesive. In respect of NTAP, this treatment did not increase the immediate μTBS values. It means the enamel-resin bonding was not favoured, despite the high hydrophilicity of both SBU and enamel surface (Liu *et al*., 2016).Thus, the first null hypothesis was accepted. Based on the present results and to the best knowledge about the dense crystalline structure and mineral composition of enamel, the authors suggest NTAP is not able to remove calcium phosphate from enamel enough to create microporosities and to promote a higher μTBS (as those well-documented for phosphoric acid etching) (Van Meerbeek *et al*., 2011).Besides, other study showed insignificant change in Ca/P ratio of enamel when plasma was applied on surface (Lehmann *et al*., 2013).

So, the effect of this treatment in enamel is quite different from the benefit observed in dentin (Abreu *et al*., 2016; Dong *et al*., 2015; Han *et al*., 2014; Dong *et al*., 2013; Lehmann *et al*., 2013).Favourable effect of NTAP for dentin/composite resin interfaces (Lehmann *et al*., 2013),the increased μTBS to dentin (Dong *et al*., 2015; Dong *et al*., 2013) as well the superficial changes induced by plasma (30 s) in dentin previously exposed to sodium hypochlorite which improved bond strength for a self-etch adhesive system (Abreu *et al*., 2016) were demonstrated. Recently, a scanning electron microscopy analysis showed NTAP increased hydrophilicity of the dentin surface (compared to the untreated one), favoured the primer penetration and led a longer and more abundant resin tags (Han *et al*., 2014).On the other hand, NTAP produced no apparent micro-morphological changes on dentin surface comparable to acid etching (Stasic *et al*. 2019). To conclude, NTAP treatment is considered unnecessary for enamel bonding. The phosphoric acid etching is still the best strategy to improve bond strength of universal adhesives to enamel.

CONCLUSION

It was concluded that the phosphoric acid etching of enamel increased the immediate bond strength of the universal adhesive, whereas non-thermal atmospheric plasma did not increase bond strength. After 12 months of storage in water, the bond strengthsignificantly reduced in all groups.

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