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Erosive cola-based drinks affect the bonding to enamel surface: an *in vitro* study

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ABSTRACT

Objective: This study aimed to assess the impact of *in vitro* erosion provoked by different cola-based drinks (Coke types), associated or not with toothbrushing, to bonding to enamel. Material and Methods: Forty-six bovine enamel specimens were prepared and randomly assigned into seven groups (N=8): C- Control (neither eroded nor abraded), ERO-RC: 3x/1-min immersion in Regular Coke (RC), ERO-LC: 3x/1-min immersion in Light Coke (LC), ERO-ZC: 3x/1-min immersion in Zero Coke (ZC) and three other eroded groups, subsequently abraded for 1-min toothbrushing (EROAB-RC, EROAB-LC and EROAB-ZC, respectively). After challenges, they were stored overnight in artificial saliva for a total of 24 h and restored with Adper Single Bond 2/Filtek Z350. Build-up coronal surfaces were cut in 1 mm^2-specimens and subjected to a microtensile test. Data were statistically analyzed by two-way ANOVA/Bonferroni tests (α=0.05). Failure modes were assessed by optical microscopy (X40). The interface of the restorations were observed using Confocal Laser Scanning Microscopy (CLSM). Results: All tested cola-based drinks significantly reduced the bond strength, which was also observed in the analyses of interfaces. Toothbrushing did not have any impact on the bond strength. CLSM showed that except for Zero Coke, all eroded specimens resulted in irregular hybrid layer formation. Conclusions: All cola-based drinks reduced the bond strength. Different patterns of hybrid layers were obtained revealing their impact, except for ZC.

Keywords: Tooth abrasion. Bonding agent. Enamel. Erosion. Soft drinks.

INTRODUCTION

Dental erosion is a common problem in modern societies, owing to the increased consumption of acidic drinks, such as soft drinks, sport drinks, fruit juices, and fruit teas, which in turn have a high potential to provoke dental demineralization[3,8,12]. Up to now, most clinical reports are generally related with a later intervention, in which the non-carious lesions as erosion, especially in cervical area of the tooth, present dentin exposure, hypersensitivity, and more complex restorative needs[6,15,17,23,24].

Erosion is a superficial demineralization process that softens the surface with subsequent wear until reaching dentin[3,9,17,23]. Substrate compromising depends on the etiologic agent and intensity of challenge[3,9,14,17,23]. As erosion is normally associated with other non-carious lesions such as abrasion, its sole cause in dental substrates is difficult to establish, since erosion consists of lesions from multiple etiologies, which may result in the need for a restorative procedure[3,6,12,15,24].

Although enamel is considered a simple and safe substrate for bonding[13,14], there is lack of

research regarding this property to eroded tooth. As erosion provokes alterations on this substrate, details of these modifications on bonding turn the investigations necessary. A few reports[17] presented an early intervention, restoring enamel. As these substrates will be subsequently restored, mostly using adhesive materials, this interaction needs to be clarified.

Thus, this investigation aimed to test the impact of different Coke drinks on adhesion to previously-eroded enamel surfaces. The null hypotheses are that (1) different Coke drinks do not provoke differences on adhesion to enamel, and (2) their association with toothbrushing does not have any impact on the adhesion process either.

MATERIAL AND METHODS

Experimental design

This experiment was conducted considering two factors: Erosive challenges by soft drinks (in four levels: none/artificial saliva, RC, LC, and ZC) and toothbrushing effect (in two levels: none or following erosive challenge). The response variable was based on bond strength.

Preparation of the specimens

Fifty-six enamel specimens (4x4x2 mm) were obtained from freshly-extracted bovine incisors, which were previously stored in 0.1% thymol solution at room temperature. One specimen was cut from each crown using an Isomet Low-Speed Saw cutting machine (Buehler, Lake Bluff, IL, USA) and two diamond disks (Extec Corp., Enfield, CT, USA), which were separated by a 4 mm thickness spacer. The enamel surface was flat with water-cooled carborundum discs (#320, 600, and 1200 of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA), resulting in enamel removal of about 100 µm depth. This series was completed with polishing using felt paper made wet by diamond spray (1 µm; Buehler, Lake Bluff, IL, USA). In order to standardize the enamel surfaces, they were selected using a micro-hardness test by performing five indentations in different regions of the block (Knoop diamond, 25 g, 5 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan). Enamel blocks with a Knoop hardness number ranging from 320 to 385 KHN were selected.

Erosive protocols

Composition and chemical characteristics of each immersion media are presented in Figure 1. Selected specimens were randomly assigned into seven groups (n=8) according to immersion media and toothbrushing association or not as presented in Figure 2.

Restorative procedures

After the erosive/abrasive challenge, each specimen was carefully cleaned under a deionized water flow for 2 min. Acid etching was performed using 37% phosphoric acid (Dentsply Ind. Com. Ltda, Petrópolis, RJ, Brazil) for 15 s, which was washed out for a 30 s. A gentle air-stream was used to promote water evaporation, which was completed with absorbent paper. Two thin coats of an etch-and-rinse dentin bonding system (Adper Single Bond 2- 3M ESPE, St. Paul, MN, USA) were subsequently dispensed with a disposable microbrush and gently air-dried for 2–5 s to allow solvent evaporation and followed by light curing for 10 s with a 1,000 mW/cm^2 power density LED unit (Radi cal-SDI, Bayswater, Victoria, Australia). Thus, the enamel surface was restored with two layers of 2 mm thickness increments of a nano-filled A2 shade resin composite (Filtek Z350-3M ESPE, St. Paul, MN, USA) and light-activated for 20 s.

Microtensile bond strength

After 24 h of water immersion in 37°C, each

Immersion media	Composition*
Artificial saliva	1.5 mmol/L $Ca(NO_3)_2$ $2H_2O$, 0.9 mmol/L $Na_2HPO_4 2H_2O$, 150 mmol/L KCl, 0.1 mol/L $H_2NC(CH_2OH)_3$ (TRIS), 0.05 µg/mL F (NaF).
Regular Coke	carbonated water, high fructose syrup, caramel color, phosphoric acid, natural flavors, caffeine content: 23 mg/8 fl oz, very low sodium. pH=2.74; tritability=120 mL (0.1 N NaOH).
Light Coke	Carbonated water, nutmeg extract, caramel color, 24 mg/100 mL aspartame, 16 mg/100 mL potassium acesulfame, phosphoric acid, sodium benzoate, sodium citrate, natural flavors, caffeine. pH=3.00; tritability=12 mL (0. N NaOH).
Zero Coke	carbonated water, caramel color, phosphoric acid, aspartame, potassium benzoate (to protect taste), natural flavors, potassium citrate, acesulfame potassium, phenylketonurics, phenylalanine, caffeine content: 23 mg/8 fl oz, very low sodium. pH=3.08; tritability=91 mL (0.1 N NaOH).

* Based on manufacturer's information, except for pH and tritability, which were assessed by the authors

Figure 1- Composition of artificial saliva and coke beverages

restored enamel specimen was longitudinally sectioned in directions across the bonded interface using an Isomet 1000 digital saw (Buehler Ltd., Lake Bluff, IL, USA) to obtain specimens with an interface area of approximately 1 mm^2. An average of 6–8 beams *per* specimen was obtained. Each beam was attached to a modified Bencor Multi-T testing apparatus (Danville Engineering Co., Danville, CA, USA) with a cyanoacrylate resin (Super Bonder Flex Gel-Loctite, Henckel Ltda, Itapevi, SP, Brazil) and submitted to test under tension in a universal testing machine (Emic, São José dos Pinhais, PR, Brazil) operating at a crosshead speed of 0.5 mm/min. After testing, the cross-sectional area at the site of fracture was measured with a digital caliper (Mitutoyo Digimatic Caliper Series/Code 500-144, Mitutoyo Sul Americana, RJ, Brazil) to calculate bond strength in mega Pascal (MPa).

Statistical analysis

Data analysis was accomplished by the Graph Pad/Prisma statistical package (GraphPad InStat for Windows version 4.0, San Diego, CA, USA). The assumptions of equality of variances and normal distribution of errors were checked for all the variables tested. Since the assumptions were satisfied, two-way analysis of variance (ANOVA) and Bonferroni *post hoc* tests were carried out for statistical comparisons and the significance was preset to 5%.

Stereomicroscopy analysis

After bonding tests, each interface was analyzed with a stereomicroscopy 40x and was categorized according to failure as: adhesive failure (failure between the enamel and bonding layer), cohesive failure in enamel (when failure occurred predominantly in enamel) or in resin (when failure occurred predominantly in resin) or mixed failure (when two or more types were observed simultaneously).

Confocal Laser Scanning Microscopy (CLSM)

For each group, two additional specimens were prepared with half of the surface protected with nail varnish in order to maintain a control surface. After the challenges, nail varnish was removed with acetone and the specimens were restored in similar conditions as described above. However, Rhodamine B (Sigma-Aldrich Brasil, São Paulo, SP, Brazil) was added to Adper Single Bond 2 in 0.16 mg/mL as a fluorescent ingredient to be detected in CLSM[4]. Following, the specimens were cut in the middle to obtain two halves containing control and exposed specimens to analyze the interfaces with the confocal microscope (Leica TCS SPE, Leica Microsystems CMS, Mannheim, Germany) using the microscope's software (Leica Application Suite Advanced Fluorescence, Leica Microsystems CMS, Mannheim, Germany). The quality of the interfaces was analyzed by examining both halves of each specimen with 40x (each 1.0°— 1.0 mm, 1,024 pixels and 0.976 µm in resolution).

RESULTS

Bond strength means and standard deviations are summarized in Table 1 and Figure 3.

The factor erosion (type of coke drink) revealed statistical significance ($p<0.05$). In contrast, no significance was attributed to abrasion performed by toothbrushing ($p>0.05$).

	No abrasion (ERO)	Following abrasion by mechanical toothbrushing (EROAB)
Control	Artificial saliva immersion for 24 h	-----
ERO-RC	3x/1 min immersion in regular Coke. Between the erosive challenges, the specimens were exposed to artificial saliva for a total of 24 h.	3x/1 min immersion in Regular Coke plus 1 min toothbrushing abrasion (Oral B Cross Action Power, Oral B do Brasil Ltda, Rio de Janeiro, RJ, Brazil) with one drop of previously-prepared slurry (3:1 w/w toothpaste - Oral B, Oral B do Brasil Ltda, Rio de Janeiro, RJ, Brazil/deionized water). After each cycle, specimens were immersed in artificial saliva for a total of 24 h.
ERO-LC	3x/1 min immersion in Light Coke. Between the erosive challenges, the specimens were exposed to artificial saliva for a total of 24 h.	3x/1 min immersion in Light Coke plus 1 min toothbrushing abrasion as described above and immersion in artificial saliva for a total of 24 h.
ERO-ZC	3x/1 min immersion in Zero Coke. Between the erosive challenges, the specimens were exposed to artificial saliva for a total of 24 h.	3x/1 min immersion in Zero Coke plus 1 min toothbrushing abrasion as described above and immersion in artificial saliva for a total of 24 h.

Figure 2- Groups tested according to erosive/abrasive challenges

Table 1- Means and standard deviations (MPa) of eroded/abraded enamel specimens restored with resin composite

	Control	ERO-RC	ERO-LC	ERO-ZC
NO	23.92 (3.68)^{Aa}	15.38 (3.82)^{Ba}	14.18 (2.87)^{Ba}	15.90 (2.68)^{Ba}
AB		14.71 (1.98)^{Ba}	16.16 (2.37)^{Ba}	12.53 (3.93)^{Ba}

N=8

*Uppercase letters show significant differences among the erosive challenges for each abrasion condition (columns) ($p<0.05$). Lower case letters show significant differences between the association with or without abrasive challenge for each erosive condition (rows)

ERO-RC=eroded with regular Coke; ERO-LC=eroded with Light Coke; ERO-ZC=eroded with Zero Coke; NO=not abraded and AB=abraded

Table 2- Failure mode distribution according to challenge (%)

Challenge	Failure mode	Percentage		
Control	Mixed		34.62	
	Adhesive		46.15	
	Cohesive/Enamel		19.23	
	Cohesive/Resin		0.00	
		RC	LC	ZC
ERO	Mixed	52.94	58.33	33.33
	Adhesive	17.65	25.00	37.04
	Cohesive/Enamel	29.41	16.67	25.93
	Cohesive/Resin	0.00	0.00	3.70
EROAB	Mixed	71.43	46.15	42.86
	Adhesive	28.57	30.77	35.71
	Cohesive/Enamel	0.00	23.08	21.43
	Cohesive/Resin	0.00	0.00	0.00

ERO-RC=eroded with regular Coke; ERO-LC=eroded with Light Coke; ERO-ZC=eroded with Zero Coke; NO=not abraded and AB=abraded; RC=regular Coke; LC=Light Coke; ZC=Zero Coke

The control group (neither eroded nor abraded) showed significantly greater bond strength compared to all other situations ($p<0.05$). For the eroded groups, all coke drinks resulted in a significant decrease in bond strength compared to the control group ($p<0.05$); however, no differences were found among the bonding to eroded groups previously treated with any coke drinks ($p>0.05$). In the comparison of bond strength of specimens submitted to erosion to each coke drink to their respective association with abrasion, no differences were observed ($p>0.05$).

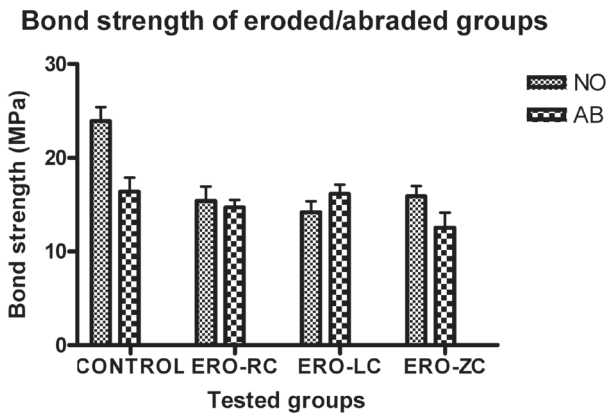

Figure 3- Bond strengths (MPa) for all tested conditions; comparing each eroded challenge in abraded and not abraded conditions

Description of distribution of the failure modes for each tested group is presented in Table 2. It could be observed that the sum of mixed and adhesive failures was evident in all conditions. Cohesive failure in resin was only present in groups eroded by ZC (3.70%). Cohesive failure in enamel was also present for all conditions, except for RC associated with abrasion.

Under CLSM observation, a control pattern in specimens (not challenged) is illustrated in Figure 4, which showed a uniform and regular tag formation, with homogeneous thickness and regular extension into enamel. On the other hand, distinct

performance was revealed comparatively regarding each coke drink that was used.

RC (ERO-RC and EROAB-RC specimens) caused superficial and heterogeneous tag formation (Figures 5a, b). It was observed that there was poor tag formation for the eroded specimens. However, for EROAB-RC specimens, even also poorly visible, tags were more regular than for ERO-RC specimens. A similar result was shown for ERO-LC, in which the adhesive impregnation was more superficial compared to control (Figures 6a, b). For EROAB-LC, the adhesive layer showed to be more regular than only eroded specimens.

Only ZC allowed similar tag formation compared to the control group, regardless of the abrasion. The interface characteristics of these groups (ERO-ZC and EROAB-ZC) can be observed in Figure 7a, b.

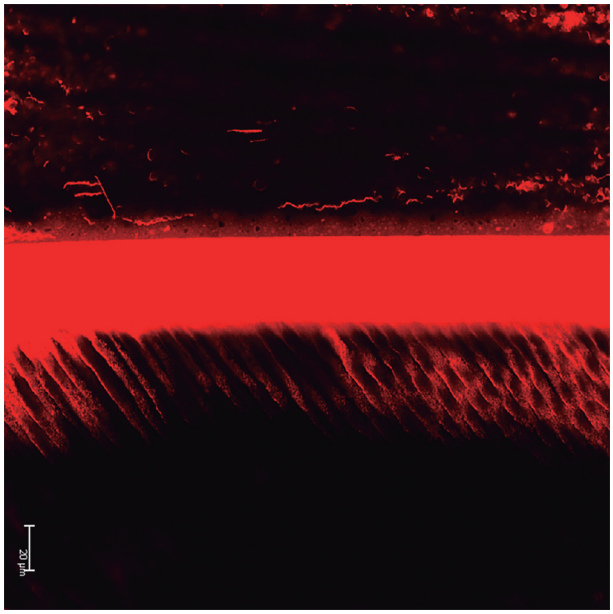

Figure 4- Interface aspect of bonding system to enamel surface (control). Tags are presented in homogeneous extent

DISCUSSION

Poor evidence is reported on the adhesion to enamel previously eroded by different soft drinks. While enamel is a dental substrate that allows the formation of regular and strong adhesion[13], changes in this substrate might affect the bond strength, failure mode, and the tag formation[1].

Based on the results of this study, the first null hypothesis was rejected while the second null hypothesis was accepted. Data attested the potential of cola-based drinks, as Coke types, to reduce bond strength in enamel compared with the control condition. As Coke drinks are based on phosphoric acid content (pH 2.6–3.0), they showed to be potentially erosive, which was previously stated[5,8,9,19,20,25]. According to Figure 1, all tested drinks presented similar pH. RC and LC present the same titratable acidity, which were higher than presented by ZC. Titratable acidity is related to the amount of base required to allow a solution with neutral pH, which exhibits relevant influence on demineralization[5,19,20,25]. Thus, it can be expected that there will be higher compromising by RC and LC. By means of bond strength, this performance

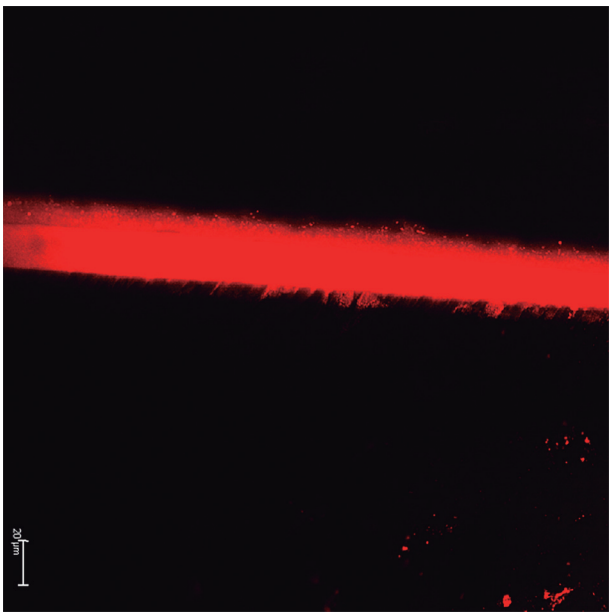

Figure 5a- Interface aspect of bonding system to enamel surface eroded by regular Coke (RC). Tags are not easily observed. Hybrid layer is based on superficial tags formation

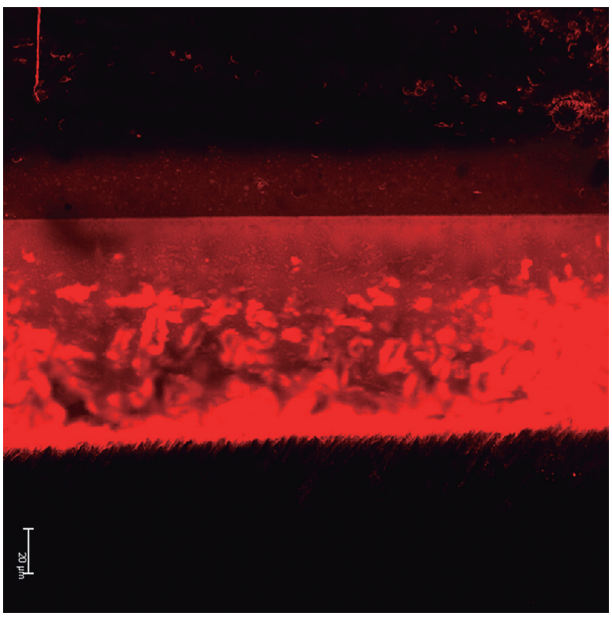

Figure 5b- Interface aspect of bonding system to enamel surface eroded by regular Coke (RC) followed by toothbrushing. Hybrid layer is irregular and superficial, but it is more evident than only eroded surface

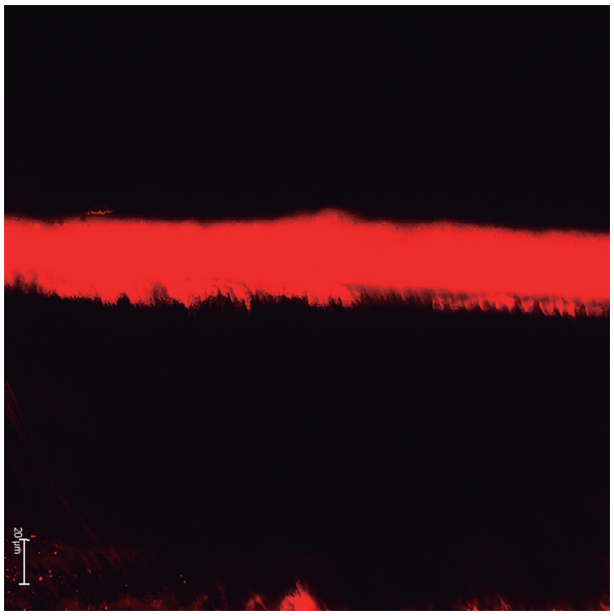

Figure 6a- Interface aspect of bonding system to enamel surface eroded by Light Coke (LC). Tags are thin and irregular

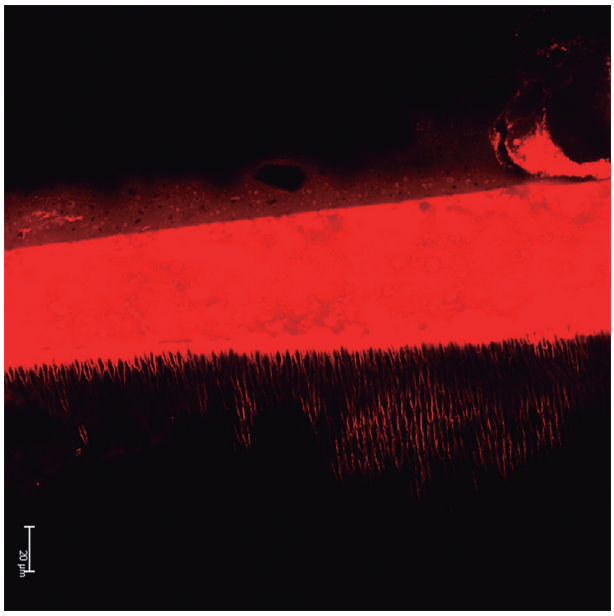

Figure 6b- Interface aspect of bonding system to enamel surface eroded by Light Coke (LC) followed by toothbrushing. Hybrid layer is also superficial, but much more regular than only eroded surface

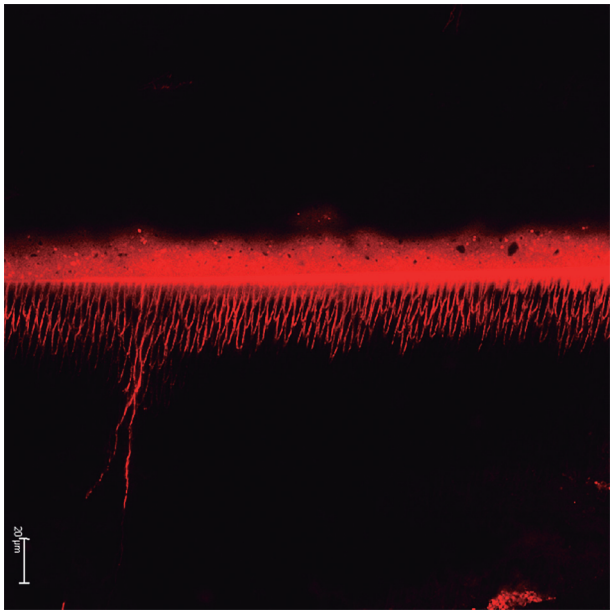

Figure 7a- Interface aspect of bonding system to enamel surface eroded by Zero Coke (ZC). Tags present homogeneous pattern

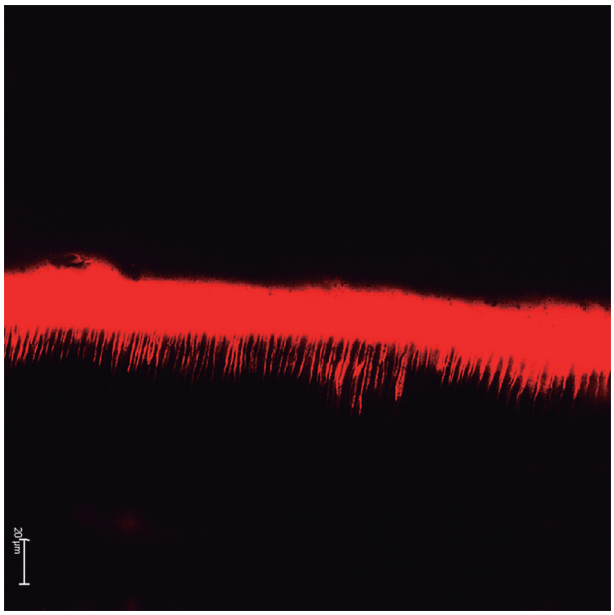

Figure 7b- Interface aspect of bonding system to enamel surface eroded by Zero Coke (ZC) followed by toothbrushing. Hybrid layer is not affected as in the control group

was not confirmed as all drinks negatively influenced adhesion with no difference among them. However, in light of the failure mode interpretation, we can observe a similar pattern of failure mode between RC and LC, with a predominant occurrence of mixed and adhesive failures, which differed from ZC (Table 2). A previous study[19] suggested that LC was less erosive than RC. This less-erosive potential was attributed to the presence of the amino acid phenylalanine, which is provided by the hydrolysis of aspartame in the presence of saliva. As the present study was conducted under the *in vitro* experimental model, there was no influence of saliva, which in turn may be responsible for the lack of differences between RC and LC.

The performance of ZC reveals that it was the only group that presented cohesive failure in resin. Likely, this beverage might provoke irregularities of

surface, which intensity was favorable to bond. A rougher surface is attributed to playing a relevant role in the adhesion mechanism, as it contributes to promoting more intense interlocking to enamel[10].

Abrasion was the other factor considered in this investigation. Early stages of enamel dissolution are accompanied by a weakening of the surface. However, the fragile enamel surface can be lost if the erosive challenge continues[9,11,18,21,22]. This softened zone is also more susceptible to mechanical forces, such as abrasion[9,11,18,21,22]. Control group, associated with toothbrushing, presented no difference in relation to respective solely eroded group. When RC was associated with toothbrushing (EROAB-RC), cohesive failure in enamel was not evident, suggesting that the eroded surface was removed by abrasion. For LC and ZC, it might be prudent to speculate that the toothbrushing has a minor impact on the enamel loss, due to the low erosive demineralization provoked by these drinks[20]. Despite the fact that some studies have shown that toothbrushing seems to have some effects on acid-softened hard tissues[18,22], the abrasion of the eroded enamel surface did not have a major impact on bond strength and failure mode in the present study.

CLSM images are in accordance with the speculated interpretation of overall results (Figures 4 to 7b). Except for ZC, all other eroded specimens presented an irregular hybrid layer formation. Both RC and LC reduced the tag formation. However, enamel eroded with RC showed more irregular interface than the LC-eroded surface. For eroded/abraded enamel specimens, the hybrid layer was more evident than for only eroded enamel, except for ZC, which was similar to the control group. We suggest that the toothbrushing abrasion partially removed the fragile enamel layer, especially in the case of RC, allowing better hybrid layer formation, even though the bond strength remained as low as that for only the eroded enamel.

Practitioners should be aware when they restore enamel erosion lesions of patients with resin-based materials, as this property seems to be somehow negatively affected. However, once more it is important to state that this investigated potential did not consider the influence of saliva and its components present in the oral environment, which could alter the results[2,7,16].

Clinical investigations have shown some concern about eroded-tooth restoration; however, most of them regard the adhesion failures in dentin[23,24]. In the present study, we focused on early treatment strategies for dental erosion which has not reached dentin. Clinically, the findings of the present study are relevant for restoring enamel erosive lesions in a facial surface of anterior teeth, which in turn might compromise the aesthetic[5]. The early adhesive restoration could also prevent the progression of enamel erosion in anterior teeth. Thus, the results of the present study highlight the bonding mechanism when enamel is involved in early stages of erosive demineralization. Furthermore, the study also showed the importance of combining different analyses to better understand the adhesion process in enamel.

CONCLUSIONS

Based on the results of this study, we can conclude that all Coke drinks reduced the bond strength no matter the type. Qualitative aspects provided more detailed information, showing different failure mode and tag formation according to the type of Coke drink. Further investigation is required to evaluate the impact of the type of Coke drink on the adhesion to enamel and also to dentin over time, using higher erosive challenges and different bonding systems.

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ERRATUM

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Due to a publishing error the article “Erosive cola-based drinks affect the bonding to enamel surface: an *in vitro* study”, published at Journal of Applied Oral Science 22(5):434-441 was printed with the following errors:

Page 434 - In abstract: Material and Methods: “Forty-six bovine enamel specimens…” should be read “Fifty-six bovine enamel specimens…”.

Page 435 – Figure 1

Light Coke	“…tritability=12 mL (0. N NaOH).”
should be read	
Light Coke	“…tritability=12 mL (0.1 N NaOH).”