# *In Vitro* Effect of Simulated Tooth Brushing and Children's Mouth Rinses on Physical Properties of Glass Ionomer Cement

Natyla ML Silva\*/ Victor G Costa \*\*/ Letícia M Gonçalves \*\*\*/ Isabella A Gomes \*\*\*\*/ Marco Aurélio B Paschoal \*\*\*\*

**Objective:** The present study investigated the erosive potential of children's mouthrinses on glass ionomer cement (GIC) samples after simulated toothbrushing. **Study design:** Forty round-shaped samples of GIC were divided into 3 groups: G1- cetylpyridinium chloride, G2- xylitol and triclosan and G3–Malva sylvestris and xylitol and G4–distilled water as a control group. Prior to the main tests, the samples were submitted to the surface roughness measurement (Ra) and weight analysis (W). Afterward, they were brushed twice day (2x / day) for 15 days and immersed in mouthrinses after the last daily brushing. The final surface roughness (R2) and weight (W2) were determined after completing the tooth brushing-mouth rinsing cycles and the real increase in roughness ( $\Delta Ra$ ) and real weight loss ( $\Delta W$ ) were calculated. In addition, stereoscopic images taken at 30X magnification. The data was analyzed by one-way ANOVA and Tukey-test post hoc tests for intergroup comparison and the T-test for dependent samples ( $\alpha = 0.05$ ). **Results:** Only group G2 showed increased in roughness  $\Delta Ra$  (1.53 ± 0.94) whereas  $\Delta W$  values were not significant. However, evident cracks and voids were verified for all tested children's rinses. **Conclusion:** Thus, children's mouthrinse containing xylitol / triclosan increased the GIC roughness, especially when associated with brushing.

Keywords: Abrasion, Mouthwash, GIC, Wear.

- \*\* Victor G Costa, Undergraduate student, Dental School, Universidade CEUMA, São Luis, Brazil.
- \*\*\* Letícia M Gonçalves, DDS, MS, PhD, Adjunct Professor, Department of Dentistry I, Federal University of Maranhão – UFMA, São Luis, Brazil.
- \*\*\*\* Isabella A Gomes, DDS, MS, PhD, Professor, Dental School, Universidade CEUMA, São Luis, Brazil.
- \*\*\*\*\* Marco Aurélio B Paschoal, DDS, MS, PhD, Adjunct Professor, Department of Child and Adolescent Oral Health, Federal University of Minas Gerais – UFMG, Belo Horizonte, Brazil.

Send all correspondence to:

Marco Aurelio Benini Paschoal

Phone: +55 31 34092496

E-mail:marcobpaschoal@hotmail.com

## INTRODUCTION

In the last few years, the use of mouthrinses has become widespread <sup>1</sup>, specially the "pediatric type", because they have a good flavor and a pleasant scent that increases their commercial attractiveness to children, who use these products without adult supervision and professional prescription <sup>2</sup>.

One of main reason for this indiscriminate use is based on the fact that these products are sold in supermarkets, pharmacies and other commercial establishments without any professional indication / supervision, making them freely available to 6-year-old and older youngsters, irrespective of the real need for this usage.

Nevertheless, the widespread use of general mouthrinses with the endorsement and publicity of large companies that do not take into consideration potential harmful health effects and possible side effects, including dental erosion and discoloration. <sup>3-6</sup>

Among the side effects, we point out wear / erosion,<sup>3,5</sup> discoloration,<sup>4,6</sup> and changes in the tooth surface (*e.g.* increasing roughness and reducing microhardness) of dental structures and materials. Moreover, for children, the risk of swallowing these products and causing fluorosis deserves priority attention.<sup>7</sup>

Trying to understand the wear mechanism is not a simple task. A previous investigation has pointed out that the potential side effects and risks of any solution (including mouthwashes) arise from the interaction of two main sources: pH (as low as 5.2 - 5.5 for enamel and 6.7 for dentin substrate)<sup>8</sup> and titratable acidity (TA).<sup>9</sup> However,

<sup>\*</sup> Natyla ML Silva, Undergraduate student, Dental School, Universidade CEUMA, São Luis, Brazil.

Department of Child and Adolescent Oral Health, School of Dentistry, Federal University of Minas Gerais–UFMG. Av. Antônio Carlos, 6627, Dental School, Campus Pampulha, Belo Horizonte, MG, Brazil 31270-901, Brazil

there is no consensus about which property is best correlated to erosive potential of acidic solutions. <sup>10</sup> As in the case of other erosive challenges, the potential of these oral products to cause some signs of wear are directly related to frequency and length of time of use. Therefore, special attention must be paid when these products are used in primary teeth, which have a smaller thickness of enamel and dentin that are subject to faster wear and loss of dental structure.<sup>11</sup> So, an important instruction, forgotten by many parents, is not to let children use these products for a long period. In addition to causing some damage to tooth surfaces, mouthwashes could affect some characteristics of restorative materials (e.g. hardness, roughness, color stability) with an possible increase in their surface degradation.<sup>9,12-15</sup>

In Brazilian pediatric dentistry practices, glass ionomer cement is a material largely used because of its versatility and some important properties, such as having anticariogenic <sup>16</sup> and fluoride releasing effects. <sup>16,17</sup> Some features such as low fracture toughness, slow setting reaction, susceptibility to surface degradation with consequent material wear, and moisture sensitivity are regarded some of the drawbacks of using this material.<sup>18</sup>

Two investigations have focused on the wear of dental structures caused by acidic beverages and mouthrinses.<sup>11,19</sup> On this topic, little is known regarding the erosive effects of children's mouthrinses on glass ionomer cement (GIC), especially when associated with the toothbrushing routine.

Hence, there is valid justification for investigating the erosive effects of children's mouthwashes after toothbrushing, as this could answer some clinical questions and enable dentists to guide patients and their parents. The purpose of this present investigation was to evaluate the erosive potential of some children's mouthrinses, combined with simulated brushing, to change the surface and weight of conventional GIC samples.

## **MATERIALS AND METHOD**

### Sample size calculation and groups

The sample size calculation was performed, based on a previous pilot study, and consisted of an 80% chance of detecting a change after erosive / abrasive cycling with a difference of 25% between the children's mouthwashes groups at 5% level of significance. Therefore, in this study, 10 samples in each group were required, consisting of a total of 40 test specimens made of a conventional high viscosity glass ionomer cement (Ketac Molar Easymix- 3M ESPE, St. Paul, USA). These were divided into 3 Groups (N = 10), according to the active principles of the studied mouthwashes, and a control group, as follows: G1-cetylpyridinium chloride (pH = 7.84; Cepacol Teen - Safoni Aventis Farmacêutica Ltda., Suzano, SP, Brazil), G2-xylitol and triclosan (pH = 6.83; Dentalclean Garfield-Rabbit Ind. Com de Prod. de Higiene Pessoal Ltda., Londrina, PR, Brazil) and G3-Malva sylvestris and xylitol (pH = 7.18; Malvatrikids Júnior-Daudt Oliveira Ltda., Rio de Janeiro, RJ, Brazil). As negative control, distilled water was used (G4).

### Sample preparation

A circular steel matrix (10 mm X 2 mm) was used to fabricate the round-shaped samples of GICs for the experimental tests. The material was inserted in a single increment and immediately afterwards, a polyester strip was placed over the GIC inserted into the matrix. A glass slide (Labor Import, Osasco, SP, Brazil) was placed on top of it, pressed down to obtain a flat surface and left until the desired setting time was reached. After fabrication, the test specimens were removed from the metal matrix, stored in artificial saliva and transferred to a bacteriological oven at  $37 \pm 1^{\circ}$ C overnight. After this period, the test specimens were submitted to the finishing and polishing technique (Soflex, 3M ESPE, Sumaré, SP, Brazil) with movements in a single direction and control of pressure, for 40 seconds. A single operator performed the procedure, in accordance with the manufacturer's instructions, to ensure surface standardization. On conclusion of the finishing and polishing technique, the specimens were randomly divided into four groups for experimental tests.<sup>12</sup>

### **Roughness analysis**

The sample roughness analyses were performed using a rugosimeter (Mitutoyo Corporation, Japan). After specimen preparation, polishing and finishing, the surface roughness (initial Ra = R1) of the samples was measured and the value expressed as the arithmetic roughness value (Ra =  $\mu$ m) was recorded. After this, the samples were submitted to the simulated brushing model and erosive cycling by exposure to the tested children's mouthrinses. On conclusion of the entire process, a new roughness analysis was performed, considered the final measurement (Final Ra = R2) with each sample being carefully dried with absorbent paper before taking the readouts.

The initial and final readout values were obtained by means of the arithmetic mean of three consecutive readouts on each test specimen to obtain the  $\Delta$ Ra value (real roughness increase). For readouts, the ISO Standard 1997 specifications were used, whereby the test specimens were submitted to readouts in a cooled room with controlled temperature and humidity. The cut-off value used was 0.8, at the speed of 0.5 mm/s.<sup>12</sup>

### Weight analysis

The samples were dried with absorbent paper and weighed in a precision scale before the simulated brushing cycles, and the initial weight value (W1; in grams) was recorded.

### **Simulated Brushing Model**

The test specimens of the four studied groups were submitted to simulated brushing in a Brushing Machine XY (BIOPDI, São Carlos, SP, Brazil) programmed for 500 cycles. This corresponded to a period of 9 months of brushing with a total of 15.000 cycles (1000 cycles per day)<sup>13</sup>, using an mixture of toothpaste and distilled water (Colgate Palmolive–Divisão Kolynos do Brasil Ltda., Osasco, Brazil) in the ratio of 2:1 following specification ISO 14569-1. To do this, brushes with soft bristles (Colgate Palmolive–Divisão Kolynos do Brasil Ltda., Osasco, Brazil) were adapted to perform this test. Simulated brushing, using 5 ml of the mixture was performed twice a day for 2 minutes for a period of 15 consecutive days. Between brushing cycles, the test specimens were transferred to a new artificial saliva solution and stored in a bacteriological oven at 37°C.<sup>3</sup>

## **Erosive Cycling Process**

After the last daily brushing challenge, the test specimens were challenged to erosive cycling consisting of 1 minute of immersion in the tested mouthrinses, were transferred to new artificial saliva, and kept in an bacteriological oven at 37°C for a minimum period of 14

hours *(overnight)*. In addition, control test specimens followed the same steps, but immersing in distilled water instead.<sup>3</sup>

## Quantitative analysis

Immediately after the abrasive/erosive challenge, at the end of the 15th day, the samples were submitted to a final roughness measurement (Final roughness = R2) and to obtain the  $\Delta$ Ra value ( $\Delta$ Ra = R2 – R1; real roughness increase). For final weight values, the samples were weighed to obtain the final weight value (Final weight = W2) and calculate the  $\Delta$ W value ( $\Delta$ W = W2 – W1; real weight loss).

## Qualitative analysis

After 15 days of the challenges described, the test specimens were evaluated by means of images captured with a stereoscopic microscope (Stereo Microscope Kozo Optical and Electronic Instrumental, Najing, China) and analyzed at 30x magnification to describe the surface characteristics.<sup>14</sup>

## Statistical analysis

First, normality of the data was tested using the Shapiro Wilk test (p < 0.05). As the sample had normal and homogeneous distribution, the Analysis of Variance (ANOVA) followed by the Tukey-test were used for intergroup comparison of the initial and final roughness and weight values. Accordingly, for intragroup comparison of both outcomes, the T-test for dependent samples was used. For all tests, the level of significance was set at 5%. For sample size calculation and statistical analysis, the software program used was SPSS for Windows, version 23.0 (IBM, Armonk, NY, USA).

## RESULTS

The data of this study were evaluated quantitatively by inferential statistics (roughness and weight values). The roughness values (R1 and R2) on the 15<sup>th</sup> day are described in the Table 1, and so are the  $\Delta$ Ra values recorded after the erosive cycling with the tested children's mouthwashes.

As may be seen, final roughness values of samples submitted to xylitol / triclosan and cetylpyridinium chloride showed an increase

in roughness. However, only Group G2 showed statistically significant difference (p = 0.01) relative to the real increase in roughness value ( $\Delta Ra = 1.53 \pm 0.94$ ).

Table 2 illustrates the weight analysis. When comparing W1 with W2, all tested groups lost weight, with exception of the control group (G4). However, when verifying the real weight loss ( $\Delta$ W), no statistical difference was verified for all mouthrinses tested when compared with the control group (P values: G1 = 0.7; G2 = 0.38 and G3 = 0.19).

Furthermore, the effect of all active principles tested on the sample surfaces illustrated by sterosocopic images showed more cracks and voids for Group G1 (cetylpyridinium chloride–Figure 1A); Group G2 (xylitol/triclosan – Figure 1B) with more surface damage in comparison with G3 (*Malva sylvestris* and xylitol – Figure 1C); and with absence of this appearance of wear for Group G4 (distilled water – Figure 1D).

## DISCUSSION

The present study investigated the erosive potential of three different children's mouthrinses containing cetylpyridinium chloride, xylitol/triclosan and *Malva sylvestris* /xylitol as active principles combined with a simulated brushing challenge.

It is well known that the degradation of dental materials is a complex process involving chemical and mechanical mechanisms, especially in the oral environment. Therefore, it was rational to perform the abrasive-erosive cycling as proposed here to achieve the aim of this study.

After the test time period, only Group G2 was found to have a higher  $\Delta Ra$  when compared with the control group, but without statistical significance when the  $\Delta W$  was investigated as well (Table 1 and 2). We could infer that the property of pH could influence these results, since the pH of xylitol/triclosan showed that it was the most acidic mouthrinse (pH = 6.83).<sup>5</sup> Accordingly, solutions with lower pH values contain higher amounts of H<sup>+</sup> ions and these attack the cement matrix, causing release of ions and consequently dissolution.<sup>20-22</sup> Downloaded from http://meridian.allenpress.com/jcpd/article-pdf/44/5/342/2645515/i1053-4628-44-5-342.pdf by Bharati Vidyapeeth Dental College & Hospital user on 25 June 202:

Table 1. Values of roughness measurements of all groups	before and after the simulated brushing / erosive cycling challenge and
the real increase in roughness value ( $\Delta Ra$ ).	

Active principles tested	Roughness values			
	R1	R2	T-test P – value*	ΔRa
G1–Cetylpyridinium chloride	1.20 (0.46)***	2.14 (0.88)	P = 0.01	0.94 (0.43)
G2–Xylitol and triclosan	0.96 (0.23)	2.49 (1.0)	P = 0.01	1.53 (0.94)
G3–Malva sylvestris and xylitol	1.54 (1.9)	1.52 (0.42)	P = 0.98	-0.02 (0.27)
G4–Distilled water	0.87 (0.15)	1.24 (0.23)	P = 0.01	0.37 (0.18)
ANOVA P-value**	P = 0.15	P = 0.01		P = 0.01
ANOVA <i>P-value**</i> Stattistical difference between groups	G1-G4 p=0.14 G2-G4 p=0.92 G3-G4 p=0.98	G1-G4 p=0.04 G2-G4 p=0.01 G3-G4 p=0.82		G1-G4 p= 0.08 G2-G4 p= 0.01 G3-G4 p=0.36

\*T-test was applied to verify differences within the same group after 15 days; P value set at 0.05.

"ANOVA test applied to investigate differences between groups after 15 days; P value set at 0.05.

\*\*\* Standard deviation values.

Active principles tested —	Weight values			
	W1	W2	T-test P-value*	ΔW
G1 – Cetylpyridinium chloride	0.24 (0.05)***	0.22 (0.04)	P = 0.16	-0.02 (0.42)
G2–Xylitol and triclosan	0.24 (0.05)	0.21 (0.03)	P = 0.08	-0.03 (0.59)
G3– <i>Malva sylvestris</i> and xylitol	0.26 (0.05)	0.24 (0.05)	P = 0.44	-0.02 (0.53)
G4–Distilled water	0.28 (0.04)	0.28 (0.04)	P =0.21	0 (0.0)
ANOVA P-value**	P = 0.23	P = 0.01		P = 0.22
ANOVA <i>P-value**</i> Statistical difference between groups	G1-G4 p=0.28 G2-G4 p=0.28 G3-G4 p=0.80	G1-G4 p=0.01 G2-G4 p=0.01 G3-G4 p=0.17		G1-G4 p=0.70 G2-G4 p=0.38 G3-G4 p=0.19

Table 2. Values of weight measurements	of all groups before and after the simulated brushing <i>i</i>	/ erosive cycling challenge and the
real weight loss value ( $\Delta P$ ).		

T-test was applied to verify differences within the same group after 15 days; P value set at 0.05.

"ANOVA test applied to investigate differences between groups after 15 days; P value set at 0.05.

\*\*\* Standard deviation values.



Figure 1. Stereoscopic image of all groups at 30 X magnification. A. Cetylpyridinium chloride group. B. Xylitol and triclosan group. C. Malva sylvestris and xylitol group. D. Distilled water group.

With regard to the model applied in this study, we could assume a possible synergistic effect (or so called supra-additive) between the abrasive effect of bristles and erosive characteristics of chemical compounds, especially those of the children's mouthrinse that contained xylitol / triclosan. In conjunction, these factors worsened the material wear with consequent influence on weight loss, corroborating the two findings.<sup>23,24</sup>

In terms of the mechanism of action, while the abrasive action could attack the GIC polyalkenoate matrix so that it became a softened surface exposing more fillers, the contact with mouthwashes would be able to dissolve the matrix, resulting in cracks and voids illustrated by the stereoscopic images, in comparison with the control group (Figure 1). Moreover, since the ionomeric material used has a complex process of absorption and disintegration, acidic degradation via immersion in the mouthrinse, could affect its water absorption, resulting in internal propagation through the matrix, filler interfaces, pores, and other defects, accelerated by a low pH and causing filler-matrix debonding (known as "*leaching effect*").<sup>25</sup> As a clinical consequence, this concomitant action resulted in higher surface roughness that led to a surface more prone to bacterial accumulation. This in turn, would lead to difficulty with dislodging oral biofilm, since the increase in Ra value to over 0.2 um would render

the surface susceptible to increased plaque accumulation and higher risk for caries development.  $^{\rm 26}$ 

The abrasive effect of brushing GIC samples before the erosive attack produced a surface with increasingly changed topography (i.e. higher roughness values) and loss of weight, as has been verified by other investigations. <sup>23,24,27,28</sup> When Sadaghiani et al (2007) <sup>29</sup> and da Silva et al (2018)<sup>5</sup>, compared the results found after different materials, including conventional GIC (CGIC) had come into contact with children's mouthrinses alone, they showed roughness values varying of 0.49<sup>29</sup>-1.67<sup>5</sup> with a preponderant effect on GIC groups when compared with composites; in contrast with Ra values of 1.52-2.49 verified in the present study. Furthermore, Yu et al (2009)<sup>23</sup> and Carvalho et al (2012)<sup>28</sup> combining erosive + abrasive tests using HCl and a demineralization and remineralization solution respectively, verified that the groups submitted to combined challenges reached substantial roughness values compared with the isolated tests. Thus, the hypothesis that both factors (abrasion + erosion) could contribute to surface damage was confirmed.

The progression of erosive defects is influenced by many factors, including type and frequency of exposure to acid, saliva buffering capacity, flow rate and composition. <sup>30</sup> The role of the latter factor could explain the weight results, since there was no statistical difference between the studied groups, results that were in agreement with those of a recent investigation that found a lower enamel surface loss when specimens were exposed to artificial saliva.<sup>31</sup> This fluid is able to dilute, clear and neutralize the acids, providing calcium and phosphate to reduce the rate of demineralization and enhance remineralization.32 In the present study, samples were kept in artificial saliva between the erosion/abrasion cycles and overnight throughout the entire period of testing. Hence, the mineral deposition and type of saliva could affect the weight samples of all the studied groups, re-hardening the softened surface layer of the material; and being artificial, it had greater remineralization potential when compared with human saliva. 33

In the present investigation, soft bristles <sup>34</sup> and Colgate Total (RDA <sup>35</sup> – relative dentin abrasion: 70) toothpaste were used, the same components as those used by many investigations <sup>24,35-37</sup>, whereas Yu *et al* (2009) <sup>23</sup> used Elmex (RDA 30), both considered to have low abrasivity. <sup>38</sup> While Oral B (RDA 117)–classified as having high abrasivity–was used by da Silva *et al* (2014). <sup>39</sup> All of these toothpastes were used on different restorative materials

and with diverse study models. Factors such as the abrasive type, particle size and dilution proportion are considered the main factors of tooth abrasion, while the toothbrush acts as a carrier, taking into consideration the number, stiffness, and shapes of tufts and bristles. <sup>40</sup> Furthermore, the combined effect of bristles and material abrasion was considered lower than the iabrasivity of paste and roughness values *per se.*<sup>41,42</sup> Thus, although the paste was considered *soft* (by RDA values <sup>35</sup>) the joint action of paste abrasivity X bristle actions X mouthrinse pH property could explain the values found for all studied groups, as stated in two studies <sup>23,24</sup> especially for triclosan/ xylitol mouthrinse.

With regard to study limitations, two aspects must be highlighted. The samples were submitted to circular movements, a load of 50g (pressure between 0.125 - 0.625N)<sup>43</sup>, 2 minutes of brushing twice a day,<sup>5</sup> at 500 cycles per session (with a total of 15.000 cycles on conclusion)<sup>13</sup> were the parameters used. They were adapted with the aim of simulating a brushing performed by a child with the support of saliva action, to simulate the clinical situation to the maximum extent, in accordance with other studies <sup>23,44</sup>. Nevertheless, comparison with other studies was a difficult task. It should be noted that this study performed fewer episodes of erosion and abrasion (only 15 days, 2X/day) with only 1 minute of "acid exposure" (considered a weaker erosive challenge) and not including other erosive challenges practiced by children, such as eating acid foods and consuming soft drinks, which does not allow for direct comparisons with clinical situations.

The important key message of the outcomes resides in alerting parents / guardians about the fact that children's mouthrinses should be used strictly according to proper guidance provided by dentists (e.g. caries risk assessment). Furthermore, this information represents an extra factor to put pressure on the manufacturing sector to indicate and highlight the effects of these chemical substances by labeling the products properly.

#### CONCLUSION

Overall, the results clearly showed that the children's mouthrinse containing xylitol / triclosan increased the material surface roughness value, and highlighted the added effect of the brushing action. Thus, parents and guardians must be aware of the potential roughness of glass ionomer cement that may occur when young children use the mentioned mouthrinse.

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