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Susceptibility of fluorotic enamel to dental erosion-abrasion

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Abstract: Dental hard tissue conditions can be of pre- or post-eruptive nature, such as enamel fluorosis and erosive tooth wear (ETW), respectively. Dental enamel fluorosis is caused by the chronic and excessive intake of fluoride during enamel development, leading to increased fluoride concentration and increased porosity. ETW has become a common clinical condition and often impairs dental function and aesthetics. This in vitro study tested the hypothesis that fluorotic enamel presents different susceptibility to dental erosion-abrasion. It consisted of a 3×3×2 factorial design, considering a) fluorosis severity: sound (TF0), mild (TF1-2), moderate (TF3-4); b) abrasive challenge: low, medium, and high; and c) erosive challenge: yes or no. A total of 144 human teeth were selected according to the three fluorosis severity levels (n=48), and subdivided into six groups (n = 8) generated by the association of the different erosive and abrasive challenges. Enamel blocks (4×4 mm) were prepared from each tooth and their natural enamel surfaces subjected to an erosion-abrasion cycling model. After cycling, the depth of the lesions in enamel was assessed by profilometry. ANOVA showed that the three-way and two-way interactions among the factors were not significant ($p > 0.20$). Enamel fluorosis level ($p=0.638$) and abrasion level ($p = 0.390$) had no significant effect on lesion depth. Acid exposure caused significantly more enamel surface loss than water ($p < 0.001$). Considering the limitations of this in vitro study, fluorosis did not affect the susceptibility of enamel to dental erosion-abrasion.

Keywords: Fluorosis, Dental; Tooth Erosion; Tooth Abrasion, Dental Enamel.

Introduction

Excessive exposure to fluoride during the enamel formation period can lead to dental fluorosis. Studies have shown that enamel with fluorosis is hypomineralized and exhibit higher fluoride content.[1-3] Fluorotic enamel is more porous, which lowers its resistance to mechanical wear; but also rich in fluoride, which could potentially increase its resistance to demineralization.[4] Fluoride compounds can reduce tooth dissolution and, in some cases, increase tooth resistance to erosive acids.[5] Clinically, the first signs of enamel fluorosis present as thin white striae across teeth surfaces, which follow the perikymata

pattern. In mild fluorosis, the cusps tips, incisal edges, and marginal ridges may be completely opaque, a sign called "snow capping." In moderate cases, the white lines appear more pronounced and may merge to produce cloudy areas scattered over the surface. As severity increases, the entire tooth surface exhibits opaque cloudy areas mixed with areas of brownish discoloration and there may be pitting.[6] There is a need to carry out a differential diagnosis with other enamel lesions such as molar incisor hypomineralization, other localized forms of developmental hypomineralization not involving molars/incisors, and enamel caries lesions.[7] Depending on the type and severity of the enamel defect, it can sometimes go unnoticed, however, enamel fluorosis can negatively affect the quality of life of some individuals due to aesthetic problems.[8] In addition, the higher surface roughness and structural defects (pores and pits) can increase adhesion to cariogenic bacteria.[9]

Erosive tooth wear (ETW) is a multifactorial condition that has received increasing attention in recent decades[10] and affects different age groups.[11] ETW develops as a consequence of excessive exposure of dental surfaces to intrinsic or extrinsic acids that are not from bacteria origin. Intrinsic ETW can be associated to anorexia nervosa, bulimia nervosa, and conditions with frequent regurgitation of gastric acids, while extrinsic ETW relates mainly to specific diets,[11,12] environmental conditions, medications and changes in lifestyle and nutritional habits. The exposure to acids causes the enamel surfaces to soften and become more susceptible to abrasive wear, leading to progressive loss of dental hard tissue.[13] Previously published systematic reviews[14,15] have reported higher levels of ETW and the predicted percentage of adults presenting with severe tooth wear increased from 3% at the age of 20 years to 17% at the age of 70 years. Increasing levels of tooth wear were significantly associated with age.[11] Although some in vitro studies have been conducted to test whether higher fluoride content in fluorotic enamel would protect it from demineralization due to caries simulation, there are no studies reporting if fluorotic enamel would present different susceptibility against dental erosion-abrasion.

This in vitro study tested the hypothesis that despite its fluoride content, fluorotic enamel of different severities is more susceptible to the development of dental erosion-abrasion lesions, when compared to sound enamel.

Methodology

Experimental design

This was a quantitative, laboratory, transversal and experimental analytical study with direct observation technique.[16] It followed a 3×3×2 factorial design, using an erosion-abrasion simulation model. The experimental factors were a) degree of fluorosis severity, at 3 levels: sound (TF0), mild (TF1-2), moderate (TF3-4); b) abrasive challenge, at 3 levels: low, medium, high; and c) erosive challenge at 2 levels: yes, no. The erosion-abrasion simulation was performed for 10 days. Human enamel specimens were selected, prepared and randomly assigned to each of abrasive-erosive levels stratified by fluorosis severity, generating the 18 groups (n=8) from the three experimental factors. The response variable was natural enamel surface loss, measured in micrometers using profilometry.

Sample size calculation

The sample size calculation was based on a previous study,[17] testing the susceptibility of fluorotic enamel to caries demineralization in vitro. A Hedge G effect magnitude of 1.375 was estimated between the most disparate groups (TF 0 and TF 3-4) reported. Using a power of 80%, significance level of 5% and one-tailed directionality, a sample size of 8 samples per group was calculated.

Teeth selection

A total of 144 unidentified extracted permanent human teeth were collected, including 48 sound (control) teeth and 96 teeth with different fluorosis severity levels. They were examined using a stereomicroscope at 2× magnification. Those with the presence of a visual caries lesion, restorations, enamel fissures / cracks, enamel fractures were excluded. The teeth were carefully cleaned using a periodontal curette to remove soft tissue. Enamel

fluorosis was visually assessed by the Thylstrup-Fejerskov Index (TFI) by a previously trained and calibrated examiner.

Specimen preparation

Enamel blocks (4×4×2 mm) were cut using a microtome (Isomet Low Speed Saw, Buehler). The specimens were embedded in acrylic resin (VariDur® Power and Liquid, High Performance Mounting System, manufactured by Buehler) to facilitate handling and positioning in the automatic brush machine for the study. A central area (2×4 mm) on the enamel surface of the specimens, was exposed to the experimental treatments. The sides of this area were partially protected with nail polish (Avon True Color Pro + Nail Enamel). After the specimens were prepared, they were assigned to the experimental groups using balanced randomization according to TFI scores.

Erosion-abrasion and abrasion cycling

Specimens in groups assigned to the erosion-abrasion cycling model were fully immersed in a 1% citric acid solution (pH ~2.4) for 5 min, without agitation. Then, the specimens were immersed in artificial saliva for 60 min, under gentle agitation, and at room temperature. [18] The specimens were positioned in an automated custom-made toothbrushing machine [18,19] and brushed with the respective abrasive slurries for 15 s (45 back-and-forth movements) using medium stiffness toothbrushes (Soft Oral-B 40, Procter & Gamble, Cincinnati, USA) and a load of 150 g. After brushing, the specimens were rinsed with deionized water for 10s and were stored in artificial saliva in a 150 rpm agitation during the night period[18] (Figure).

The slurries were prepared by mixing different abrasives: higher (Zeodent 103/15%; RDA [standard-deviation]: 208.0 [9.96]), medium (Zeodent 124/10%; RDA: 146.9 [9.96]) and lower (Zeodent 113/5%; RDA: 69.2 [9.96]; Zeodent, J.M. Huber, Etowah, USA) with 0.5% carboxymethycellulose solution.[20]

Profilometric analysis

The depth of simulated lesions was analyzed by optical profilometry (Proscan 2000 profilometer,

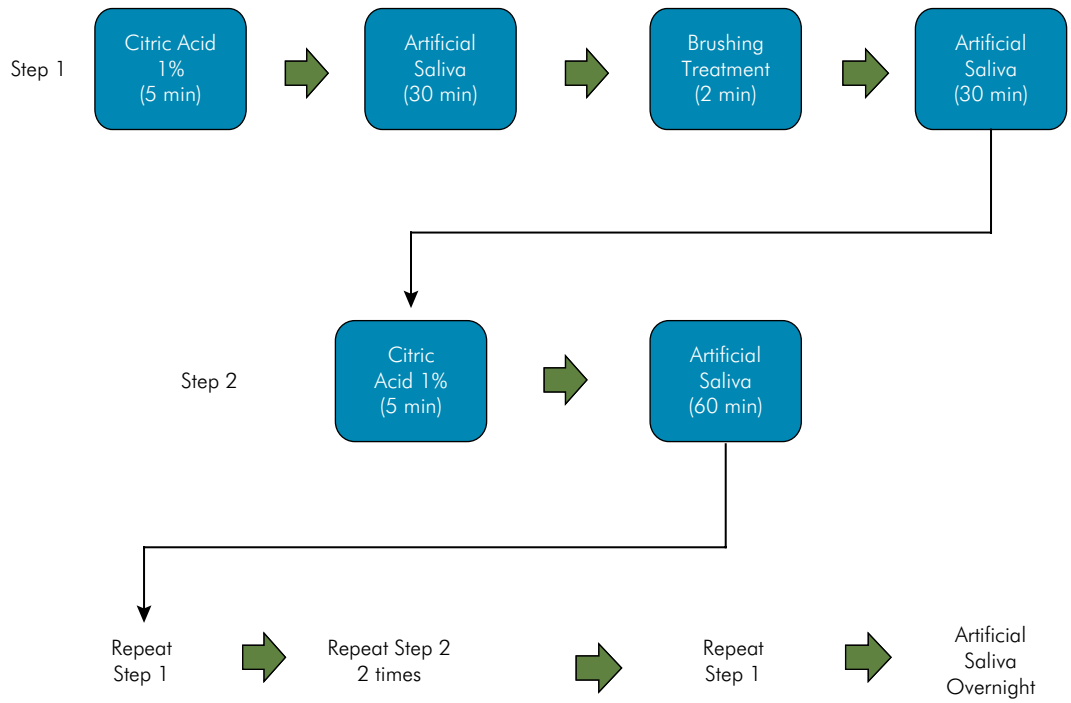

Figure. Daily sequence of erosion cycling and erosion-abrasion.

Scantron Industrial Products Ltd, Taunton Somerset TA2 8DE, England), before and after simulations. Scans were performed using the S65/10 (10 mm; vertical resolution of 0.30 mm) sensor, with X = 0.03, 180 steps; Y = 0.03, 180 steps and analyzed using dedicated software (Proscan 2000 software, V2.1.1.10, Scantron). The step height was calculated based on the difference in the surface profiles obtained before and after the erosive challenge, similarly to the procedure reported previously.[21,22]

Statistical analysis

Three-way ANOVA was used to evaluate the effects of fluorosis level (TF0, TF 1-2, TF 3-4), erosion simulation (acid, water), and abrasion level (low, medium, high) on height loss. Due to non-normality and heterogeneous variances, analyses used the ranks of the data to satisfy ANOVA assumptions. A two-sided 5% significance level was used for all tests. Analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, USA).

Results

Descriptive statistics on step height are shown in Table 1. The three-way and two-way interactions among the factors were not significant ($p > 0.20$, Table 2). Enamel fluorosis level ($p = 0.638$) and abrasion level ($p = 0.390$) had no significant effect on height loss. Acid exposure had significantly more height loss than water ($p < 0.001$).

Table 1. Descriptive statistics of step height values (mm) for the experimental groups.

Erosion	Abrasion	N	Mean	SD	SE	95% CI	Min	Max
Sound	Yes							
	High	8	92.1	22.0	7.8	73.7–110.4	45.1	114.8
	Medium	8	86.7	14.5	5.1	74.5–98.9	60.9	98.5
	Low	8	85.9	14.2	5.0	74.0–97.9	58.0	101.2
	No							
	High	8	12.3	4.9	1.7	8.2–16.4	7.6	20.6
	Medium	8	13.2	6.4	2.3	7.8–16.5	6.0	24.0
	Low	8	15.0	13.3	4.7	3.8–26.1	5.1	47.1
TF 1-2	Yes							
	High	8	97.3	22.6	8.0	78.4–116.2	46.0	120.0
	Medium	7	93.0	12.8	4.8	81.2–104.8	70.8	106.2
	Low	8	77.3	23.1	8.2	58.0–96.6	47.7	105.0
	No							
	High	8	11.4	6.5	2.3	6.0–16.8	4.8	24.2
	Medium	8	15.0	5.1	1.8	10.7–19.3	6.8	21.6
	Low	8	10.7	4.9	1.7	6.6–14.7	3.4	20.2
TF 3-4	Yes							
	High	8	90.5	21.2	7.5	72.8–108.3	69.5	126.0
	Medium	7	86.6	41.9	15.8	47.8–125.3	32.1	156.5
	Low	8	93.0	37.8	13.4	61.4–124.6	33.1	169.2
	No							
	High	8	17.4	7.5	2.7	11.1–23.7	4.2	27.3
	Medium	8	18.0	12.0	4.2	7.9–28.0	6.8	42.3
	Low	8	21.8	29.6	10.5	-2.9–46.5	2.5	93.4

Table 2. ANOVA Table.

Effect	Num DF	Den DF	F-Value	p-value
Fluorosis level	2	124	0.45	0.638
Erosion	1	124	358.94	<.001
Fluorosis level * Erosion	2	124	1.54	0.219
Abrasion	2	124	0.95	0.390
Fluorosis level * Abrasion	4	124	0.68	0.607
Erosion *Abrasion	2	124	0.79	0.455
Fluorosis level * Erosion * Abrasion	4	124	0.40	0.811

Discussion

The findings of this in vitro study showed that under our experimental conditions, fluorosis, regardless of the severity level studied (mild or moderate), does not impact enamel susceptibility to dental erosive and abrasive challenges. This result is somewhat surprising, since it has been reported that the intrinsic structure of fluorotic enamel presents a higher level of porosity, when compared to sound enamel.[4] In theory, these structural differences should render enamel more susceptible to not only demineralization but also toothbrushing abrasion, as simulated in this study. A previous laboratory study has observed an increase in the susceptibility of fluorotic enamel to caries-like lesions,[17] partially supporting the proposed theory. In that investigation, enamel with more severe fluorosis (TF 3-4) was less resistant to demineralization than sound enamel (TF0), even though the fluoride concentration found in fluorotic enamel was significantly higher than that found in sound enamel. The authors attributed the higher porosity of the enamel in TF 3-4 teeth as the reason for the increased demineralization, since the diffusion of the acid into the enamel could be facilitated and the higher porosity results in a larger mineral area to be dissolved by the acids.[17]

The contrast between the findings of the present study and those of the study of Marín et al.[17] can be explained by differences in the experimental methods and underlying mechanisms (caries versus dental erosion). In the previous study, a relatively milder acid (acetic acid, pH 4.3) was used. We suggested that this less aggressive model allowed the differences between sound and fluorotic enamel to be clearly highlighted. On the other hand, the present study considered a relatively more severe erosive challenge simulation with the use of citric acid (pH 2.4), which may have been overly aggressive and not allowed the potential differences between sound and fluorotic enamel loss to be detected. Citric acid was chosen as it is commonly found in fruit juice drinks and most carbonated beverages and is generally accepted as the standard acid in several models for the simulation of dental erosion.[18,23,24] While caries lesion formation involves a slow diffusion of dissolved minerals that combine with a liquid phase partially saturated with regard to tooth mineral, frequently creating conditions for remineralization in response to demineralization events,[25] the transport of ions dissolved by demineralization is much faster in dental erosion,[26] so that mineral loss involves more intensively the enamel subsurface in caries and is more concentrated at the enamel surface in erosion. It has been shown that the higher contrast in fluoride content between normal and fluorotic enamel is found at the enamel subsurface,[3] which would favor a higher impact of fluorosis on caries (subsurface phenomenon) than in dental erosion (surface phenomenon). This is consistent with the findings of the current study.

The different abrasive level of toothpastes simulated in this study had no impact on the enamel erosion-abrasion lesions development, although some non-significant trends were observed for the higher abrasive toothpaste to cause more enamel loss in both sound and mild fluorotic enamel substrates. Nevertheless, the lack of clear impact of the toothpaste abrasivity was not expected, based on previous

reports in this area, using dental erosion-abrasion simulation models.[19] Similarly to what was suggested for the impact of the acid challenge on fluorotic enamel, the abrasive challenge may have been overshadowed by the dental erosive challenge not allowing differences on the influence of the tested toothpastes to be found. When dental erosion was not simulated, much lower values of enamel loss were observed with no clear indication of the impact of the toothpaste abrasivity on the results, regardless of the presence of fluorosis.

Considering the relatively high variation observed in the measurements, it is likely that these findings may be related to some of the limitations of the simulation model, specifically with the use of natural surfaces for the test simulation. The vast majority of laboratorial studies in the dental abrasion and erosion area use flattened and polished dental surfaces, in order to minimize the error caused by biological variation among specimens and to maximize the capability of the evaluation methods to identify differences between the experimental factors. This experimental approach was not possible in this study, as we aimed to study the natural fluorotic surfaces, which did not allow for any processing of the enamel surfaces.[23-25] It is possible that different results could have been found if fluorosis severity levels higher than those studied (TF > 4) were to be considered. This was not done in this study, as our main focus was on non-cavitated enamel surfaces. TF scores higher than 4 were therefore excluded, as they consist of enamel with pits and cavities from post-eruptive changes. Additionally, our study design followed that reported by Marin et al.;[17] who also used scores of 1-4 in fluorotic enamel.

When interpreting the relevance of the findings of this study, it is also important to consider the myriad of clinical factors that may directly or indirectly affect the dental abrasive clinical wear caused by the toothbrushing procedure associated to a toothpaste. Modulating factors such as behavior (toothbrushing frequency, pressure, length, type of toothpaste, type of toothbrush), chemical (fluoride, detergents, acid) and biological (dental substrate, saliva, and dental biofilm), as well as any interaction among them should be considered.[27] Therefore, there is a need for further studies controlling these factors in an isolated and interacting manner. Future studies in this area should also focus on reducing the experimental error, with improvement of the experimental simulation and evaluation of dental erosion-abrasion on natural enamel surfaces. Additionally, the use of a larger sample size could increase the robustness of similar types of studies.

Conclusion

Within the limitations of this in vitro study, fluorotic enamel of mild and moderate severities do not show different susceptibility to dental erosion-abrasion. Further investigations are warranted, focusing on the investigation on more severe fluorotic enamel, as well as on the improvement of experimental conditions and evaluation methods that could minimize the experimental errors observed.

Acknowledgment

This study was reviewed and approved by the Research Ethics Committee (Federal University of Paraíba - Brazil, CAAE: 23704619.2.0000.5188), and by the Institutional Review Board of the Indiana University Purdue University of Indianapolis (process # NSO 911-07).

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