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Impact of toothbrushing frequency and toothpaste fluoride/abrasivity levels on incipient artificial caries lesion abrasion

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ABSTRACT

Objectives: To investigate the interplay among brushing frequency, dentifrice slurry abrasivity, and fluoride content on the surface loss (SL) of incipient enamel caries-like lesions.
Methods: Lesions were created in 96 bovine enamel specimens (5 × 5 mm) using methylcellulose acid gel. Specimens were randomly allocated to 12 groups (n = 8), resulting from the association of three experimental factors: (1) slurry abrasive level [low: REA = 4/RDA = 69 and high: REA = 7/RDA = 208], (2) fluoride concentration [275 and 1250 ppm F as NaF], and (3) brushing frequency [1, 2 and 3× daily]. Specimens were kept in artificial saliva in between brushings and overnight. SL was determined by optical profilometry after lesion creation, 1, 3, 5, and 7 days. Data was analyzed with repeated measures ANOVA and Tukey's tests ($\alpha = 0.05$).
Results: High abrasive slurry caused significantly more SL than low with 275 ppm ($p < 0.001$) but not with 1250 ppm fluoride ($p = 0.34$). Fluoride at 275 ppm had significantly more SL than 1250 ppm with high abrasive slurry after 7 days ($p = 0.008$). Brushing 1×/day had significantly less SL than 3×/day after 7 days with high abrasive slurry ($p = 0.016$), especially in the 275 ppm fluoride groups.
Conclusions: Higher fluoride concentration increased protection against the deleterious effect of high abrasive slurry. SL was higher if brushing was performed more than twice daily especially in low fluoride groups.
Clinical significance: Highly abrasive toothpaste formulations might cause more surface wear to incipient caries lesions, especially at higher brushing frequencies. Increasing the fluoride content may be beneficial at these circumstances.

1. Introduction

Dental caries is presented at early stages as localized demineralization in the form of white spots [1]. These lesions do not require surgical intervention and can be managed by eliminating local causative factors and enhancing remineralization via fluoride products [2,3]. While this process has long been described [4], it has been argued that the arrestment of while spot lesions could be driven mostly by a mechanical process [5] rather than fluoride remineralization action [6].

Among the most commonly used fluoride delivery vehicles, dentifrices stand out as they are widely used and convenient [7]. Fluoride dentifrices also contain abrasives that are needed to provide enamel stain removal and adequate cleaning [8,9]. Incipient caries lesions tend to have a softer surface compared to sound enamel [10,11], being therefore more prone to toothbrushing abrasion. The interplay between the remineralization potential of fluorides and enamel surface abrasion has been suggested clinically. Artun and Thylstrup concluded that the gradual regression of white spot lesions could primarily be due to surface abrasion with some mineral deposition after microscopically examining enamel surface replicas [5]. Kielbassa et al. confirmed this finding and reported more surface loss associated to toothbrushing with highly-abrasive toothpastes [12]. Nassar et al. [13] further investigated this interplay in an in vitro model and concluded that fluoride provided some protective effect against toothbrushing abrasion of simulated incipient enamel caries lesions. Specifically, this effect was reported with twice-daily brushing with 275 ppm fluoride-containing slurries.

Evidence suggests that the anti-caries efficacy of fluoridated dentifrices increases with the higher frequency of use [14]. Further remineralizing and rehardening can be achieved by increasing the frequency of toothbrushing [15]. However, this is inevitably accompanied

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by more abrasive sessions that can affect the surface integrity of the lesions. This has been shown previously to increase abrasive wear due to toothbrushing [16,17].

The main objective of the present study was to further investigate how toothbrushing frequency would impact the effects of slurry abrasiveness and fluoride content on the surface loss (SL) of incipient enamel caries-like lesions.

2. Materials and methods

2.1. Experimental design

This study followed a 2 × 2 × 3 × 4 factorial design (abrasive level × fluoride content × brushing frequency × time). The null hypothesis was that there would be no interaction among the four studied experimental factors. Enamel caries-like lesions were created and brushed with slurries, simulating dentifrice-saliva mixtures containing low or high abrasive loads with two levels of fluoride (275 and 1250 ppm fluoride). A total of 96 specimens of bovine enamel were prepared and randomly distributed among the groups (n = 8). SL values were measured after lesion creation (baseline) and after days 1, 3, 5, and 7 of testing, using an optical profilometer.

2.2. Specimen preparation and lesion creation

Incipient enamel caries-like lesions were created in ninety six 5 × 5 mm bovine enamel slabs using methylcellulose lactic acid gel protocol for 7 days as described previously [13]. Reference areas were made in each specimen by applying adhesive UPVC tapes leaving a 2 × 5 mm central area exposed to the acid gel.

2.3. Surface loss measurement

Surface loss (SL) was measured using an optical profilometer (Proscan 2000, Scantron, Taunton, UK) after the creation of the lesions as well as at days 1, 3, 5, and 7. Tapes were removed from specimens and an area of 3 × 1 mm in the center of the specimen (covering both exposed and tape-covered areas) was scanned. The SL was calculated by subtracting the height of the exposed area from the two reference (tape-covered) areas using dedicated software (Proscan 2000, Scantron). Profilometric analysis readings from each treatment group were used as baseline for subsequent SL analyses after the brushing-remineralizing procedures. In addition, baseline SL measurements were used for stratified randomization of the specimens into the experimental groups.

2.4. Daily brushing-remineralizing procedure

Specimens were brushed with soft toothbrushes (Oral-B 40, Procter & Gamble, Cincinnati, OH, USA) mounted in a custom-made brushing machine, under 150 g of load [18]. Four slurry variants (Table 1) were used at two abrasive levels with 275 ppm fluoride (as NaF representing 1100 ppm F of regular toothpaste diluted at a ratio of 1:3) or 1250 ppm fluoride (as NaF representing 5000 ppm F of prescription toothpaste diluted at a ratio of 1:3). Abrasive slurries were prepared by mixing the ingredients above with an aqueous suspension containing 0.5% (w/w) Blanose 7 M F carboxymethylcellulose (CMC; Ashland, USA) and 10% (w/w) glycerol. Sixty grams of the slurry were used in each slot of the brushing machine.

Specimens (n = 8) were brushed with their respective assigned slurry for the designated frequency. Groups were brushed for 1, 2 or 3 × per day. Each session included brushing the specimens for 50 strokes (15 s). Specimens were stored in artificial saliva (1.45 mM $CaCl_2$. $2H_2O$, 5.4 mM KH_2PO_4, 0.1 M Tris buffer, 2.2 g/L porcine gastric mucin, pH 7.0) under stirring at 150 rpm between sessions and overnight. After their assigned session was performed, specimens allocated to the 1 × and 2 × groups were washed with deionized water and stored to eliminate the possible effect of fluoride while specimens of the 3 × groups were brushed. The brushing protocol was repeated for 7 days, resulting in a total of 350 (1 ×), 700 (2 ×), or 1050 (3 ×) double-strokes depending on the group.

Table 1
Abrasive slurry compositions[a].

Abrasivity	Fluoride (ppmF, as NaF)	Abrasives (g)	RDA[b]	REA[c]
Low	275	3.0 / Zeodent® 113[d]	69 (2.6)	4.01 (0.28)
Low	1250	3.0 / Zeodent® 113	69 (2.6)	4.01 (0.28)
High	275	9.0 / Zeodent® 103	208 (9.4)	7.14 (0.69)
High	1250	9.0 / Zeodent® 103	208 (9.4)	7.14 (0.69)

[a] All slurries also contained 2.5 g carboxymethylcellulose (Blanose 7 M F, Ashland, USA), 5.0 g glycerol and were made up to 60.0 g with deionized water.
[b] Radioactive dentin abrasivity [mean (standard deviation)].
[c] Radioactive enamel abrasivity.
[d] Zeodent abrasives are precipitated silica abrasives (J.M. Huber Corporation, USA).

2.5. Statistical analysis

Repeated-measure analysis of variance (ANOVA) was used to test the effects of slurry abrasiveness (low, high), slurry fluoride (275 ppm F, 1250 ppm F), brushing frequency (1, 2, 3 ×), and time (1, 3, 5, and 7 days) on SL. An unstructured variance/covariance matrix was used to model the variances and correlations within a specimen over time. Pairwise comparisons among the treatment combinations were made using Tukey's multiple comparisons procedure to control the overall significance level at 5%. The analyses were performed after a natural logarithm transformation of the data to satisfy the ANOVA assumptions.

3. Results

Table 2 shows means and standard errors of surface loss recorded for all groups with statistical testing for fluoride and abrasive effects. According to the statistical testing, we rejected the null hypothesis and concluded that there was a combined effect of the parameters studied. High F concentration (1250 ppm F) was able to significantly reduce the SL compared to the 250 ppm F, for days 5 ($p = 0.0308$) and 7 ($p = 0.008$), for the highly abrasive formulation, when used 3 × /day. No F protective effect was observed at Days 1 ($p = 0.77$) and 3 ($p = 0.34$), or when brushing with low abrasive formulation at 1 × ($p = 0.99$) or 2 × /day ($p = 0.68$).

Significantly more SL was recorded with high-abrasive slurries containing 275 ppm fluoride and with 3 × /day brushing frequency ($p = 0.0001$) but not with 1250 ppm fluoride-containing slurries or if brushing was carried out once ($p = 0.95$) or twice daily ($p = 0.36$). The difference between high and low abrasivity increased as the number of days increased.

Statistical testing for the effect of brushing frequency and time are shown in Fig. 1 (groups brushed with low abrasive slurries) and Fig. 2 (groups brushed with high abrasive slurries). Brushing 1 × /day had significantly less SL than brushing 3 × /day after 5 days with high abrasiveness ($p = 0.0249$) and after 7 days with high abrasiveness ($p = 0.016$) and with 1250 ppm fluoride ($p = 0.0172$). No other brushing frequency comparisons were statistically significant ($p > 0.14$).

Significantly more SL was recorded at day 7 ($p < 0.0001$) compared to SL values observed after 3 and 5 days within the protocol. For high abrasives, the group brushed at 3 × /day and with low fluoride had more SL at day 5 compared to day 1 ($p < 0.005$). A similar trend was