ORIGINAL RESEARCH

Influence of Different Mouthwashes on the Efficacy of Fluoridated Dentifrices in Prevention of Enamel Erosion: An *In Vitro* Study

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Abstract

Aim: The purpose of the current study was to evaluate the impact of three various mouthwashes on the effectiveness of fluoride dentifrices in preventing enamel erosion.

Materials and methods: A total of 120 sound intact human premolar teeth which were extracted for orthodontic treatment were selected for the study. A 3 × 3 mm window section was positioned in the middle of the coronal surface of the tooth in order to define the study area. Each sample was placed in a solution of 1% citric acid (pH 3.5) for 10 minutes in order to produce an eroded surface. All samples were divided into two main groups (60 samples each) as follows: Group A for sodium fluoride dentifrices and group B for stannous fluoride dentifrices, again it is subdivided into: CHX: Chlohex ADS®, EO: Listerine®, CPC: Colgate® Plax (20 samples in each subgroup). After that, samples underwent the pH cycling model for 5 days. Samples were examined for surface loss using a scanning electron microscope.

Results: In sodium fluoride dentifrices group, before intervention, the surface loss was 3.12 ± 1.03 in CHX group, 3.08 ± 1.20 in EO group, and 3.09 ± 0.96 in CPC group. After intervention, the less surface loss found with CHX group (2.18 ± 0.84), followed by CPC (2.34 ± 0.74) and EO group (2.46 ± 0.97). In stannous fluoride dentifrices group, before intervention, the surface loss in CHX group was 3.26 ± 1.19, in EO group, it was 3.18 ± 1.31, and in CPC group, it was 3.22 ± 1.06. After intervention, the less surface loss found with CHX: group (1.90 ± 0.54), followed by CPC (2.24 ± 0.28) and EO group (2.38 ± 0.20).

Conclusion: The present study concluded that the fluoride dentifrices' preventive effects against tooth surface loss were unaffected by a different mouthwashes with varying compositions and major constituents. In terms of erosion, fluoridated toothpaste containing stannous fluoride was found to provide better surface loss protection than sodium fluoride.

Clinical significance: Primary prevention and the eradication of contributing causes are the greatest strategies for preventing erosion. Simultaneously, antibacterial agent in the mouthwashes may help in enhancing the effect of fluoride in the enamel, owing to their high affinity for teeth structures. Therefore, in addition to cause-related treatment, further efforts to reduce tooth tissue loss are also necessary.

Keywords: Enamel erosion, Fluoride dentifrices, Mouthwash, Scanning electron microscope.

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Introduction

Dental erosion is the irreversible loss of tooth structure that can result from an interaction between non-bacterial acids. The causes of tooth erosion are numerous and can be either intrinsic (frequently brought on by gastric reflux disease, bulimia, and rumination) or extrinsic (diet, particularly soft drinks; acidic drugs; and industrial or environmental toxins).[1]

Early enamel erosion results in the loss of the enamel's mineral composition, which leads to enamel softening. Mechanical power can quickly wear away the softened enamel, which is less resistant to physical force. As a result, enamel structure loss will manifest later in the erosion process. Numerous clinical issues, including dentin hypersensitivity and poor aesthetics, can be brought on by dental erosion. Teeth can become shorter and the vertical dimension of the occlusion can be lost as a result of a severe form of dental erosion.[2]

Primary prevention and removing the causes of the erosion are the best ways to stop or prevent it. Fluoride is well-known for encouraging remineralization and preventing demineralization of acid-exposed tooth surfaces. As a result, fluoride has been a prime choice for research into how it can help prevent dental erosion.[3] In addition to encouraging the re-hardening of developing enamel

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erosive lesions, fluoride dentifrices have also proved successful in increasing the re-mineralized lesions' resilience to further erosive attacks.[4]

The most popular way to keep healthy teeth is to regularly brush with fluoride toothpaste and then rinse with mouthwash. Since a very long time ago, antimicrobial mouthwashes have been used to supplement standard oral hygiene practices, assisting in the treatment of gingivitis and periodontitis as well as favoring the decrease in dental cavities.[5] Commercially accessible formulations include those using cetylpyridinium chloride (CPC), essential oils, and chlorhexidine gluconate (CHX), among others. Although some mouthrinses may erode enamel because of their low pH, it is unknown to what extent these mouthrinses can modulate the effect of fluoride derived from toothpaste in an erosive-abrasive model. Mouthrinses may dissolve fluoride that is bonded to teeth and reduce the impact of the anti-erosive ingredients in toothpaste.[6] However, due to their strong affinity for dental structures; mouthwashes that contain an antibacterial ingredient may aid in boosting the effect of fluoride on the enamel and also limited literature available on these mouthwashes efficacy with fluoridated dentifrices. Hence, the current study was conducted to evaluate the impact of three various mouthwashes (CHX, EO, CPC) on the effectiveness of fluoride dentifrices in preventing enamel erosion.

Materials and Methods

The present study was conducted in the department of Periodontics, Kalinga Institute of Dental Sciences, Bhubaneswar, India, during the year 2022. A total of 120 sound intact human premolar teeth which were extracted for orthodontic treatment were selected for the study. With 6% hydrogen peroxide, the teeth were cleaned and kept in saline.

Preparation of Samples

Each selected tooth underwent comprehensive ultrasonic cleaning before being polished using a polishing brush and rubber cup. After the samples had air-dried, a window section measuring 3 × 3 mm was placed in the middle of the tooth's coronal surface to help define the study area. The remaining portion was given a coat of nail polish and allowed to dry. The samples were prepared and kept until the experiment in distilled water.

Preparation of Eroded Lesion

Each sample had a 3 × 3 mm center exposed region, which was submerged in 1% citric acid (pH 3.5) for 10 minutes. Using agitation and a pH meter that had been calibrated, the pH of the solution was determined. By adding sodium hydroxide solution, the pH of the solution was increased from natural to 3.5, and the volume was adjusted with deionized water (DIW) to 1 L. The blocks were submerged for 10 minutes, then gently removed from the liquid, thoroughly rinsed with deionized water for 10 seconds and dried.

Sample Allocation

All samples were mainly divided into two groups (60 samples each) as follows:

Group A: Sodium fluoride dentifrices (20 samples in each subgroups)

- CHX: Chlohex ADS® Mouthwash (Group Pharmaceuticals Ltd, India).
- EO: Listerine® Mouthwash (Johnson & Johnson, India).
- CPC: Colgate® Plax Mouthwash (Colgate-Palmolive, India).

Source of support: Nil
Conflict of interest: None

Table 1: Evaluation of the impact of three different mouthwashes on the sodium fluoride dentifrices in prevention of enamel erosion

Mouthwashes	*n*	*Before intervention [Mean ± SD (µm)]*	*After intervention [Mean ± SD (µm)]*
CHX: Chlohex ADS	20	3.12 ± 1.03	2.18 ± 0.84
EO: Listerine	20	3.08 ± 1.20	2.46 ± 0.97
CPC: Colgate Plax	20	3.09 ± 0.96	2.34 ± 0.74
F value		9.038	7.834
p-value		0.964	0.082

Group B: Stannous fluoride dentifrices (20 samples in each subgroups)

- CHX: Chlohex ADS® Mouthwash (Group Pharmaceuticals Ltd, India).
- EO: Listerine® Mouthwash (Johnson & Johnson, India).
- CPC: Colgate® Plax Mouthwash (Colgate-Palmolive, India).

Baseline enamel surface loss was evaluated for each sample before intervention. Then samples were then put through the pH cycling model for 5 days, where the samples were treated with a dentifrices containing either sodium fluoride or stannous fluoride twice daily. Remineralization process was carried out using artificial saliva, which contained calcium chloride, 0.44 gm/L; 2 gm/L; potassium chloride; 1.40 gm/L; sodium carboxymethyl cellulose; 0.50 gm/L; sodium bicarbonate; and 0.06 gm/L of magnesium chloride, along with enough distilled water and phosphoric acid to bring the pH to 6.4. The amount of enamel surface erosion was assessed once the 5-day cycle was completed.

Evaluation of Samples Using Scanning Electron Microscope

After being air-dried, mounted on aluminum stubs with carbon tape, and sputter-coated with a 5 nm thick gold/palladium alloy for conductivity, representative samples from each group were examined. The samples were chemically characterized using SEM (Zeiss sigma, Germany), and the image was captured at a magnification range of x2000. To prepare the samples for inspecting the surface changes, the samples were placed in the scanning electron microscope and vacuum was produced. Enamel photomicrographs were taken, and the surface loss were evaluated.

Statistical Analysis

SPSS software (version 20.0) was used to compute the mean and standard deviation. With the help of one-way analysis of variance (ANOVA), the various groups were compared with one another. A *p*-value less than 0.05 was used to determine the statistical significance.

Results

Table 1 presents the impact of three different mouthwashes on the sodium fluoride dentifrices in the prevention of enamel erosion. Before intervention, the surface loss was 3.12 ± 1.03 in CHX group, 3.08 ± 1.20 in EO group, and 3.09 ± 0.96 in CPC group. After intervention, the less surface loss found with CHX group (2.18 ± 0.84),

Table 2: Evaluation of the impact of three different mouthwashes on the stannous fluoride dentifrices in prevention of enamel erosion

Mouthwashes	*n*	*Before intervention [Mean ± SD (μm)]*	*After intervention [Mean ± SD (μm)]*
CHX: Chlohex ADS	20	3.26 ± 1.19	1.90 ± 0.54
EO: Listerine	20	3.18 ± 1.31	2.38 ± 0.20
CPC: Colgate Plax	20	3.22 ± 1.06	2.24 ± 0.28
F value		10.172	8.218
p-value		0.964	0.001

Table 3: Evaluation of overall mouthwashes on the stannous fluoride dentifrices in prevention of enamel erosion

Groups	*Comparison with*	*Mean difference (I–J)*	*Sig.*
CHX: Chlohex ADS	EO: Listerine	–0.48	0.001
	CPC: Colgate Plax	–0.34	0.001
EO: Listerine	CHX: Chlohex ADS	0.48	0.001
	CPC: Colgate Plax	0.14	0.092
CPC: Colgate Plax	CHX: Chlohex ADS	0.34	0.001
	EO: Listerine	–0.14	0.092

followed by CPC (2.34 ± 0.74), and EO group (2.46 ± 0.97). No statistical difference was found between the mouthwash groups.

Effect of three different mouthwashes on the stannous fluoride dentifrices in the prevention of enamel erosion is depicted in Table 2. Before intervention, the surface loss in CHX group was 3.26 ± 1.19, 3.18 ± 1.31 in EO group and 3.22 ± 1.06 in CPC group. After intervention, the less surface loss was found with CHX group (1.90 ± 0.54), followed by CPC (2.24 ± 0.28), and EO group (2.38 ± 0.20). There was a statistically significant difference found between the mouthwash groups.

On overall comparison of mouthwashes on the stannous fluoride dentifrices in the prevention of enamel erosion reveals that the significant difference found between CHX and EO, CHX and CPC, and there was no significant difference found between EO and CPC (Table 3).

The inference of the study includes that the different mouth rinses with different compositions and main ingredients did not affect the protective actions of fluoridated dentifrices on tooth surface loss. Concerning dental erosion, better protection against surface loss was seen with fluoridated toothpaste containing stannous fluoride compared with sodium fluoride.

Discussion

Erosive tooth wear, a global public health issue, can lead to underlying demineralization of the afflicted tooth tissue as well as crater formation on the tooth surface. As a result, both the restoration of the eroded tooth surface and the remineralization of the demineralized tissue are necessary for the control of erosive tooth wear (ETW). Because restoring the damaged tooth surface with restorative or esthetic procedures is expensive, ETW must be controlled with simple, cost-effective measures. Fluoride compounds, such as HAP, CPP-ACP, CPP-ACP-F, and several self-assembling peptides, have been very successful at remineralizing demineralized tissue but not at replacing lost tissue. Certain proteins have demonstrated tremendous promise for the formation of scaffolds that control the mineralization of organized calcium phosphate crystallites.[7,8]

The amount of calcium and phosphate ions present in saliva or biofilm affects the fluoride uptake. Fluorapatite [$Ca_{10}(PO_4)_6F_2$] is composed of 10 calcium ions, 6 phosphate ions, and 2 fluoride ions in one unit cell. In order to supersaturate the saliva and aid in remineralization, topical treatments of calcium and phosphate complexes, such as dentifrices, release minerals like Ca^{2+} and PO_4^{3-} ions together with fluoride ions under an acid challenge.[9]

Surface roughness or loss measurement is a method for calculating erosion. The profilometry, microradiography, scanning electron microscopy, atomic force microscopy, nano and microhardness tests, and iodide permeability tests are the most often used methods for examining the erosively changed dental hard tissues.[10] The current study has chosen scanning electron microscopy as its method of measuring of surface loss because its accuracy.

In the current investigation, the group using stannous fluoride dentifrices demonstrated noticeably more protection of enamel surfaces than the group using sodium fluoride dentifrices. As demonstrated by O'Toole S et al.[11] using basic erosive wear examination and Paepegaey AM et al.[12] using profilometry, SnF_2 provided greater protection to enamel surfaces than NaF after erosive cycles. The present study results contrast with the study of Dehghan et al.,[13] where author described enamel loss depth. Enamel loss was significantly higher in groups where the toothpaste was used. Additionally, the enamel surface erosion was considerably reduced after 60 minutes of contact to artificial saliva.

The current study examined the impact of several mouthwashes on the fluoride-containing toothpaste's surface loss protection. The findings of the study revealed that none of the tested mouthwashes (CHX, EO, and CPC) demonstrated a statistically significant difference in terms of fluoride evaluation for their capacity in surface loss from erosion in the group of dentifrices containing sodium fluoride. After brushing with fluoridated toothpaste, the study samples were immediately given mouth rinses, which increased the fluoride release from the samples and decreased their effectiveness. The results of the study by Duckworth et al.[14] were comparable to those of the current investigation, showing that using fluoride toothpaste alone does not provide nearly as much protection against erosion as using mouthwash after brushing.

Due to the development of amorphous deposits on the enamel surface and the integration of Sn ions into damaged enamel, stannous fluoride has been shown to improve acid resistance.[6] Iliana Diamanti et al.[15] reported that there was no discernible difference between NaF and SnF_2, which is contrary to our findings. Additionally, an *in situ* investigation by West NX et al.[16] revealed that NaF toothpaste outperformed SnF_2 in reducing dentin surface loss following erosive cycles. SnF_2, however, has higher ability to lessen dentin erosion and abrasion, as demonstrated by research by Ganss et al.[17]

The present study's limitation was that it was conducted in *in vitro* and did not consider the soft tissue and oral mucosa in an *in vivo* environment, which represents the real erosive settings. On the tongue, fluoride and other actives including CHX, CPC, and EO may be maintained. This could change their interaction in addition to increasing their retention due to its vast surface area. However, more research is required to understand the morphological alterations of tooth substrates under *in vivo* circumstances.

Conclusion

Within its limitations, the present study concluded that the fluoride dentifrices' preventive effects against tooth surface loss were unaffected by a different mouthwashes with varying compositions and major constituents. In terms of erosion, fluoridated toothpaste containing stannous fluoride was found to provide better surface loss protection than sodium fluoride.

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