ROLE OF MOUTWASHES ON FLUORIDE DENTIFRICES IN PREVENTION OF DENTAL ABRASION OR EROSION- AN IN VITRO STUDY

Dr. Bharti Dua[1], Dr. Mani Baweja[2], Dr. Sonia Datta[3], Dr. Anupam Bhardwaj[4]
[1]Senior Lecturer, Department Of Prosthodontics, Santosh Dental College, Ghaziabad. E-mail:
[2]Senior Consultant, Clove Dental, Delhi.
[3]Professor, Department Of Public Health Dentistry, Inderprastha Dental College & Hospital, Sahibabad.
[4]Senior Lecturer, Department of Oral & Maxillofacial Surgery, Santosh Dental College, Ghaziabad

CORRESPONDING AUTHOR:
Dr. Bharti Dua
Senior Lecturer Department of Prosthodontics Santosh Dental College

ABSTRACT
Objectives: To investigate and compare the impact of chlorhexidine gluconate (CHX), essential oils (EO) or cetylpyridinium chloride (CPC) on Erosive Tooth Wear (ETW) protection afforded by conventional fluoride toothpastes.
Methodology: A clinically relevant in-vitro erosion/abrasion pH cycling model was employed to test the effect of the aforementioned rinses on modulating the ability of NaF and SnF2 toothpastes.
Results: The mean dentin surface loss associated with NaF toothpaste was significantly lower than for SnF2 toothpaste. Enamel surface loss with SnF2 toothpaste was found to be significantly lower than for the NaF toothpaste. Also, the surface loss of erosion when associated with abrasion was significantly higher than without brushing and for both enamel and dentin. There was no significant difference in the surface loss among all mouthwashes.
Conclusion: Commonly used mouthwashes containing antimicrobial agents or additional fluoride, do not impact fluoride toothpaste action on erosion/abrasion. SnF2 dentifrice provided better protection against surface loss of enamel than the others.
Keywords: Abrasion, Erosion, Fluoride toothpaste, Mouthwash

Introduction

Tooth wear increases by 50% with the combined effect of erosion and abrasion. The softened enamel is vulnerable and can be easily removed by physical action.[1] Proper diagnosis may stop the progression of erosion considering patients comply with the dental consultation. The best approach to prevent or stop ETW is primary prevention and elimination of causative factors.[2] Therefore, along with cause-related treatment, supplemental measures to minimize tooth wear are also essential.[3]

Fluoride has long been known to promote remineralization and prevent demineralization of teeth surfaces subjected to acids.[4] Consequently, fluoride has been an obvious candidate for assessing its potential to aid in prevention of dental erosion.[5] Fluoride dentifrices have been effective in not only promoting re-hardening of incipient enamel erosive lesions but also causing the increased resistance of the re-mineralized lesions to any future erosive attack.[6] It has been shown that the presence of Sodium Fluoride (NaF:1,100-ppm) in dentifrices could reduce dentin wear by erosion and abrasion; however, this protective effect does not increase with higher fluoride concentration dentifrices.[7] Stabilized stannous fluoride (SnF2) dentifrices are unique among over-the-counter dentifrices because there are indications that the presence of both ions is relevant for erosion prevention.[8] The mechanism of the stannous and fluoride ions in erosion prevention seems to be related to the formation of a thin layer on the enamel surface, composed of different precipitates such as Sn2(PO4)OH, SnF3PO4, Ca(SnF3)2, and CaF2.[9]

Regular toothbrushing with fluoridated toothpaste followed by rinsing with mouthwashes is the most common method to maintain good oral hygiene. Use of antimicrobial mouthwashes augments the routine oral care measures by helping the treatment of gingivitis and periodontitis and to favour the reduction of dental caries.[10] A variety of formulations are commercially available, such as those containing chlorhexidine gluconate (CHX), essential oils (EO) or Cetylpyridinium chloride (CPC). Although some mouthwashes may cause enamel erosion because of their low pH, it is unknown to what extent they modulate the effect of fluoride derived from toothpaste. Mouthwashes may dissolve tooth-bound fluoride and reduce the effect of the toothpaste delivered anti-erosive agents.[11]

METHODOLOGY

In this study, an established erosion/abrasion model was employed to investigate the impact of CHX, EO and CPC mouthwashes on ETW protection accorded by two conventional fluoride toothpastes differing in fluoride compound. The present study followed a 4 (treatment rinses incl. controls) × 2 (fluoride toothpastes) × 2 (erosion with and without toothbrushing abrasion) factorial design. These factors were tested in both enamel and dentin substrates and analysed independently. Test rinses were CHX, EO, CPC, a fluoride rinse (positive control), and deionized water (negative control); fluoride toothpastes were SnF2 or NaF-containing ones. Bovine enamel and dentin specimens were subjected to a 5-day pH cycling model with twice-daily treatments, with or without abrasion, with fluoride toothpaste, followed by exposure to mouthwashes. Erosion was performed five times daily. After five days, the enamel and dentin surface loss were determined using non-contact profilometry and the efficacy of each treatment combination (toothpaste + mouthwash) compared.

The study investigated ETW prevention provided by two fluoride toothpastes in combination with five mouthwashes. Mouthwashes were chosen based on their popularity among dental patients, common availability in the market and likelihood of recommendation by dentists.

The two toothpastes were, NaF-toothpaste: Senquel F (Dr Reddy’s, India) and SnF2-toothpaste: (Crest (Procter & Gamble, India)

The five mouthwashes were a)CHX: Chlohex ADS® Antiseptic and Antiplaque Mouthwash. Active Ingredients: Chlorhexidine Gluconate 0.2 percent (Group Pharmaceuticals Ltd, India) b) EO: Listerine® Original Mouthwash. Active Ingredients: Eucalyptol 0.092 percent, Menthol 0.04 percent, Methyl salicylate 0.060 percent and Thymol 0.064 percent (Johnson & Johnson, India) c) CPC: Colgate® Plax Complete Care Mouthwash. Active Ingredients: Cetylpyridinium Chloride 0.075 percent, Sodium Fluoride .05% (Colgate-Palmolive, India) d) F: Listerine® Cavity Fighter Mouthwash. Active Ingredients: Eucalyptol 0.092 percent, Menthol 0.04 percent, Methyl salicylate 0.060 percent, Thymol 0.064 percent and NaF (Johnson & Johnson, India) e) D/W: Distilled water as negative control group.

The abrasive level of the test toothpastes was determined using the radioactive dentin abrasivity (RDA) method, as described in ISO11690. Specimens were brushed in a custom-made automated toothbrushing machine with suspensions (n = 8) prepared with the testing toothpastes (20 g in 45 ml deionized water) or with the standard calcium pyrophosphate (Ca2P2O7) abrasive material (15 g in 55 ml of an aqueous solution of 0.5 percent carboxymethylcellulose and 10 percent glycerine). After each brushing run, a 1-ml sample of the suspension was put on a liquid scintillation counter for radiation detection, expressed in counts per minutes (cpm)/gram of suspension. The net cpm/gram of the standard abrasive was assigned a value of 100, and the RDA values of the testing dentifrices were calculated considering their cpm/gram values in relation to the standard abrasive.

Specimen Preparation

Enamel and dentin slabs measured (5 mm width × 5 mm length × 2 mm thickness), and stored in 0.1 percent thymol solution pH (7.0) at 4°C were prepared. Enamel slabs were obtained from middle third of bovine maxillary incisors, crowns, and dentin slabs were obtained from bovine mandibular incisors roots.

The bottom and topsides of the enamel and dentin slabs were sequentially ground flat. Next, the slabs were cleaned with deionized water in an ultrasonic chamber for a duration of 5 min. Following that, slabs were encased in acrylic resin blocks (DPI Acrylic, India) utilizing a custom-made silicon mould, leaving the enamel and dentin surfaces uncovered. The encased blocks were ground and polished with silicon carbide grinding paper followed by 1-μm diamond polishing suspension.

Two encased specimens were adhered together to form the study block. During the exposure to toothpastes, the blocks were kept in the toothpaste slurry with only one of the sides being exposed to the brush action. The study blocks were then randomly allotted to 10 experimental groups with eight specimen blocks per group (n = 8). Adhesive unplasticized polyvinyl chloride (UPVC) tapes were attached to the surface of the specimens, leaving an about 1 × 4 mm uncovered in the centre of each of the enamel and dentin slabs.

The daily treatment regimen consisted of two treatments, with or without toothbrushing, with the study toothpastes as aqueous slurries, followed by the assigned rinse treatment after brushing, five acid challenges with a citric acid solution and exposure to artificial saliva at all other times (Table 1).

Table 1- Treatment steps with duration

	TREATMENT	DURATION
STEP 1	Erosion of specimen on account of Citric acid	5 min
STEP 2	Remineralization achieved with artificial saliva	60 min
STEP 3	Exposure done to fluoride toothpaste slurry with the help of brushing machine (only 1 side brushed for abrasion)	15 sec (45 strokes)
STEP 4	Exposure done to treatment mouthwash	1 min
STEP 5	Remineralization achieved with artificial saliva	60 min
STEP 6	Erosion of specimen on account of citric acid	5 min
STEP 7	Remineralization achieved with artificial saliva	60 min
STEP 8	Erosion of specimen on account of Citric acid	5 min
STEP 9	Remineralization achieved with artificial saliva	60 min
STEP 10	Erosion of specimen on account of Citric acid	5 min
STEP 11	Remineralization achieved with artificial saliva	60 min
STEP 12	Erosion of specimen on account of Citric acid	5 min
STEP 13	Remineralization achieved with artificial saliva	60 min
STEP 14	Exposure done to fluoride toothpaste slurry with the help of brushing machine (only 1 side brushed for abrasion)	15 sec (45 strokes)
STEP 15	Exposure done to treatment rinse	1 min
STEP 16	Remineralization achieved with artificial saliva	Overnight

An automated brushing machine was used. Specimens were brushed twice daily for 45 strokes/15s each (OHRI brushing machine) with Oral-B toothbrushes (Procter & Gamble, India) using 150 g of load with one of the two types of toothpaste. Toothpaste slurry was prepared by mixing 120 g toothpaste with 360 g distilled water.

After toothbrushing, specimens were subject to mouthwash treatments for 1 min under gentle agitation (50 rpm; orbital shaker). After the last cycle each day, the specimens remained in artificial saliva in a closed container at room temperature until the next day. After the study was finished, surface loss (SL) was calculated with the help of an optical Profilometer. The tapes were removed and the specimen was positioned in the optical profilometer with the experimental surface parallel to the horizontal plane. An area of 2 ×1 mm covering both reference areas (previously protected with UPVC tapes) and treated (exposed) surfaces was scanned using horizontal resolutions of 0.01 and 0.05 mm, in the x and y directions, respectively. Dentin specimens were allowed to dry for 10 min before scanning, in order to reduce the possible interference caused by the shrinkage of the dentin organic content. Images were analysed using Proscan 2000 (Scantron), which calculates the height of a pair of reference areas and subsequently subtracts it from the relevant area. The difference in the depth (surface loss), expressed in micrometre, was the response variable in this study. Separate analyses were performed for the dentin and enamel data. The effects of rinse (5 levels), toothpaste (2 levels), and toothbrushing (2 levels) on surface loss were analysed using ANOVA. Pair-wise comparisons between treatment combinations were made using the Sidak method to control the overall significance level at 5 percent. The distribution of the surface loss measurements was examined and a transformation of the data (e.g. natural logarithm) were used to satisfy the ANOVA assumptions.

RESULTS

The RDA data of the test toothpastes can be found in (Table 2). The SnF_2 containing toothpaste was found to be more abrasive than the NaF-containing toothpaste ($p < 0.0001$). The surface loss of dentin and enamel that was exposed to erosion with abrasion was significantly higher than without abrasion ($p < 0.0001$).

Table 2- Relative Dentine Abrasion

TEST ARTICLE	RELATIVE DENTINE ABRASION
Senquel F	146.56 ± 10.35
Crest	100.93 ± 2.16

DENTIN

There was no interaction among the three factors (type of toothpaste slurries, mouthwashes types and brush/ not brush; $p = 0.0520$). The mean (SD) dentin surface loss (μm) for NaF toothpaste treated specimens was significantly lower than for SnF_2 toothpaste treated specimens ($p < 0.0001$). The dentin surface loss was not significantly different among rinse types ($p = 0.9927$).

ENAMEL

There was no interaction among the three factors (type of toothpaste slurries, mouthwashes types and brush/ not brush; $p = 0.4720$). The mean (SD) enamel surface loss (μm) for NaF toothpaste treated specimens was significantly higher than for SnF_2 toothpaste treated specimens (p <

0.0001). The enamel surface loss was not significantly different among rinse types (p = 0.1946).

DISCUSSION

In this study, an established five-day erosion/abrasion cycling protocol was employed, involving episodes of erosion challenges, remineralization in artificial saliva, brushing abrasion and mouthwash treatments.

For the erosive challenge, we used 0.3 percent citric acid (pH 2.6) five times per day for five minutes each time. Artificial saliva containing mucin was applied between erosive and abrasive challenges for one hour as well as for overnight storage.

This allowed for the adsorption of mucin onto the eroded specimen surfaces, thus modulating the remineralization process in a similar manner as human saliva. The 1-hour saliva storage was designed to simulate the pellicle layer that remains on tooth surfaces just after brushing, and constant bathing in artificial saliva enabled the maturation of the pellicle over time.

Each specimen was brushed for 30 seconds, the equivalent of 15 seconds or 45 brushing strokes for each surface, with the toothbrushes attached to brushing machine. The toothpaste slurries were prepared using commercially available fluoridated toothpastes by adding one part (120g) of toothpaste to three parts (360g) deionized water. The slabs were immersed in these solutions for one minute, two times per day. One half of enamel and dentin samples received NaF toothpaste, the other received SnF2 toothpaste. Then the specimens were subjected to mouth rinse treatments for one minute. Non-contact surface profilometry was used for analysing combined erosion-abrasion tissue loss.

The surface loss was statistically different ($p < 0.0001$) between enamel and dentin specimens that were subjected to the brushing process in comparison to the non-brushed groups. In agreement with previous findings, which showed that after five erosive cycles, SnF2 offered more protection to enamel surfaces in comparison to NaF and sodium monofluorophosphate (SMFP).[12,13] In contrast to enamel, dentin was afforded more protection against surface loss by NaF compared to SnF2.The potential of sodium fluoride to inhibit dentin erosion is attributed to the formation of F rich layer that acts as a physical barrier against acidic challenges.[14] The main result of the present study is that there was no statistically significantly difference between CHX, EO, CPC, F and D/W rinses. There was no statistical difference among all tested rinses in the surface loss results. The tested rinses were used immediately after the brushing procedure with fluoride slurry, which may have accelerated the clearance of fluoride from the tooth surface and reduce its efficacy. In the present study, mouthwash applications were conducted under 50 rpm agitation, which can lead to partial removal of loosely bound fluoride on the tooth surface.

The tested sodium fluoride mouthwash (positive control) was not statistically significant different compared to D/I water ($p = 0.9927$ for dentin, and $p = 0.1946$ for enamel). The explanation of this may be that the low fluoride concentration does not afford protection against erosion, or that the specimens had little capacity to accumulate further fluoride after treatment with toothpaste slurries. The present results showed no effect of CHX on the anti-erosive action of fluoride dentifrices. This is probably because of fluoride clearance from the enamel and dentin surfaces due to rinsing action, which reduce the F retention. However, studies on caries and using inherently different outcome measures contradict the present findings: an in-vivo study conducted in 1994 found that the combination of CHX and fluoride was significantly more effective in reducing both lesion depth and mineral loss.

The present findings for EO and CPC rinses match those for CHX in that no significant difference was found between these mouthwashes and other controls in their ability to modulate the effect of fluoride dentifrices in ETW prevention. Our results are in agreement with a previous study that showed no statistically significant difference between EO and water after the fifth cycle of erosion.

Also, the present study was conducted in vitro and did not take into account the soft tissue and oral mucosa, in a vivo environment, which reflects the actual erosive conditions. Fluoride and other actives, such as CHX, CPC and EO, may be retained on the tongue. Due to its large surface area, this may not only increase their retention but also alter their interaction, which requires further research.

Moreover, the time interval between brushing and rinsing was kept constant which may not necessarily be representative as some rinses (CHX) are recommended to be used at least 1 h after toothbrushing. In future studies, different waiting times between brushing and rinsing should be considered.

CONCLUSION

Mean dentin surface loss associated with NaF toothpaste was significantly lower than for SnF2 toothpaste. On the other hand, enamel surface loss with SnF2 toothpaste was significantly lower than for the NaF toothpaste. Also, the surface loss of erosion when associated with abrasion was significantly higher than without brushing and for both enamel and dentin. There was no significant difference in the surface loss among all mouthwashes. Within the limitations of the present study, we concluded that commonly used mouthwashes containing antimicrobial agents or additional fluoride, do not impact fluoride toothpaste action on erosion/abrasion. Also, considering erosion only, the tested SnF2 dentifrice offered greater protection against enamel surface loss than the tested NaF dentifrice. For dentin, considering erosion only, the tested NaF dentifrice offered greater protection against surface loss than SnF2 dentifrice. Toothbrushing abrasion of previously eroded enamel and dentin significantly increased surface loss.

Contributorship: All contributors on this paper meet the criteria for authorship. All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, acquisition, analysis, interpretation, writing, and revision of the manuscript.

Conflict of interest: The authors declare that there is no conflict of interest.

Funding: The author(s) received financial support for the research, authorship, and/or publication of this article.

REFERENCES

1. Eisenburger M, Shellis RP, Addy M. Comparative study of wear of enamel induced by alternating and simultaneous combinations of abrasion and erosion in vitro. Caries Res 2003;37(6):450-5.
2. Lussi A, Hellwig E. Risk assessment and preventive measures. Monogr Oral Sci 2006;20:190-99.
3. Attin T, Becker K, Roos M, Attin R, Paque F: Impact of storage conditions on profilometry of eroded dental hard tissue. Clin Oral Investig 2009;13(4):473-8.
4. White DJ, Nelson DJ, Faller RV. Mode of action of fluoride: application of new techniques and the methods to the examination of the mechanism of action of topical fluoride. Adv Dent Res 1994;8(2):166-74.
5. Huysmans MC, Young A, Ganss C. The role of fluoride in erosion therapy. Monogr Oral Sci 2014; 25:230-43.
6. Zero TD, Hara AT, Kelly SA, et al. Evaluation of a desensitizing test dentifrice using an in situ erosion remineralization model. J Clin Dent 2006; 17(4):112-6.
7. Magalhães AC, Rios D, Moino AL, Wiegand A, Attin T, Buzalaf MAR. Effect of different concentrations of fluoride in dentifriced on dentin erosion subjected or not to abrasion in situ/ex vivo. Caries Res 2008;42(2):112–6.
8. Eversole SL, Saunders-Burkhardt K, Faller RV. Erosion prevention potential of an over-the-counter stabilized SnF2 dentifrice compared to 5000 ppm F prescription-strength products. J Clin Dent 2015;26(2):44-9.
9. Schlueter N, Hardt M, Lussi A, Engelmann F, Klimek J, Ganss C. Tin-containing fluoride solutions as anti-erosive agents in enamel: an in vitro tin-uptake, tissue-loss, and scanning electron micrograph study. Eur J Oral Sci 2009;117(4):427-34.
10. Mandel ID. Chemotheraputic agents for controlling plaque and gingivitis. J Clin Periodontal 1998;15:488-98.
11. Freitas CS, Diniz HF, Gomes JB, Sinisterra RD, Cortes ME. Evaluation of the substantivity of chlorhexidine in association with sodium fluoride in vitro. Pesqui Odontol Bras 2003;17(1):78-81.
12. O'Toole S, Bartlett DW, Moazzez R. Efficacy of sodium and stannous fluoride mouthrinses when used before single and multiple erosive challenges. Aust Dent J. 2016 Dec;61(4):497-501.doi:10.111/adj.12418.
13. Paepegaey AM. Day TN, Boulding A. Harris R, Barker ML, Bellamy PG. In vitro comparison of stannous fluoride, sodium fluoride, and sodium monofluorophosphate dentifrices in the prevention of enamel erosion. J Clin Dent 2013;24(3):73-8.
14. Ganss C, Klimek J, Schäffer U, Spall T. Effectiveness of two fluoridation measures on erosion progression in human enamel and dentine in vitro. Caries Res 2001;35:325-30.
15. Dehghan M, Tantbiroin D, Davis EK, Stewart CW, Zhang YH. Neutralizing salivary pH by mouthwashes after an acidic challenge. J Investig Clin Dent 2015; Nov 30 doi: 10.1111/jicd.12198.
16. Ullsfoss BN, Ogaard B, Arends J, Ruben J, Rölla G, Afseth J. Effect of a combined chlorhexidine and NaF mouthrinse: an in vivo human caries model study. Scand J Dent Res 1994;102(2):109-12.