

Original Article
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Title: Preventive effects of matrix-metalloproteinase inhibitors on dental erosion

Running title: Matrix-metalloproteinase inhibitors on dental erosion

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Abstract Page

Abstract

The purpose of this study was to investigate the effects of Chlorhexidine (CHX) and Epigallocatechin-3-gallate (EGCG) on dental erosion. Forty four non-carious, recently extracted human third molars were used. The 44 dental crown slabs were randomly divided into 4 groups as follow: Group 1: 1.100 ppm Sodium Fluoride (NaF), Group 2: 0.61% EGCG, Group 3: 12% CHX, Group 4: 0.2 CHX. Samples individually immersed in 30 mL of Coke® for 5 days, 4 times, 1 minute a day for erosive demineralization. The samples were immersed in 1.100 Sodium Fluoride (NaF), 0.61% EGCG, 12% CHX, 0.2% CHX for 4 times, 1 minute, each day for remineralization. The changes in the dental surface were evaluated by using a stylus profiler with surface roughness (Ra values). Topographic characteristics were assessed by Scanning Electron Microscope (SEM). Mann-Whitney U, Kruskal Wallis and Wilcoxon Signed Ranks tests were used for statistical analyses. Significant decrease was observed in the remineralization measurement of Ra values in the EGCG group when compared with the demineralization values ($p < 0.05$). SEM evaluation revealed that remineralization images of EGCG were observed more regularly than the other materials. With regard to topographically; EGCG was found as successful as CHX and NAF in terms of turning demineralized areas into remineralized areas.

Keywords: Dental erosion; Demineralization, Remineralization; Matrix Metalloproteinase; Fluoride

Main text

Introduction

Dental erosion occurs when non-bacterial chemical reactions cause dental hard tissues to permanently wear away.¹ This can be caused by extrinsic or intrinsic etiological factors. Extrinsic factors include consumption of acidic foods, soft drinks (fruit juices, sports drinks, energy drinks), medications, lifestyle, occupational exposures and environmental factors.²⁻⁴ Intrinsic factors include vomiting associated with psychological disorders such as anorexia and bulimia, gastroesophageal reflux and regurgitation.⁵⁻⁷

Fluoride prevents erosive demineralization through the formation of a layer of calcium fluoride (CaF₂), which acts as a physical barrier and mineral reservoir to prevent contact with acid attacks.^{4,8} Fluoride accelerates the remineralization process by saturating the tooth mineral.⁴ Studies have shown that fluoride provides better protection to dentine by inhibiting matrix metalloproteinases (MMP), thus slowing the progression of erosion.⁹

Chlorhexidine (CHX) is a commonly used MMP inhibitor agent which prevents demineralization. The inhibitory effect of CHX on MMPs (zinc activated, calcium-dependent endopeptidase) is dependent on a chelating mechanism, as adding calcium-chloride-binding CHX could block the inhibition of MMP-2 and MMP-9.¹⁰ CHX, which is also an inhibitor of endogenous dentin enzymes, decreases erosive wear at rates comparable to the use of fluoride and green tea extracts.¹¹

Green tea polyphenols, epigallocatechin gallate (EGCG) in particular, have been found to have inhibitory effects on MMPs.¹² EGCG binds to calcium ions to make the enamel resistant to acid and protects calcium ions to facilitate future remineralization.¹³ Studies indicate that use of EGCG is a promising preventive measure to prevent dental erosion.¹⁴ EGCG and CHX have been evaluated among inhibitors that perform on collagenolytic enzymes in situ.¹⁵ The compounds were incorporated in rinse solutions or gels for topical use. Both CHX and EGCG are classic MMP inhibitors.¹⁶

Various studies continue to be conducted on the benefits and harms of green tea and the amount of fluoride released from tea. According to the research of Maleki et al., four cups (400 mL) of tea per day and considering 2 g of tea leaves or one tea bag per cup, daily fluoride intake would vary from 0.06 g for Green Tea Leaf to 1.32 g for Green Tea Bag.¹⁷

Acid attacks that cause caries or dental erosion result in demineralization of dental hard tissue.¹⁸ Most studies report that dental erosion is limited to enamel.^{9,19,20} Enamel erosion is mainly a mineral dissolution process during which irreversible loss of tooth volume occurs, eventually resulting in dentin exposure. Exposed collagen in dentin can be degraded as a consequence of endogenous collagenases, including MMPs, even if demineralization of enamel and dentin by external acids has ceased.^{10,20} MMPs are critical actors in the erosion progression of both dentin and saliva.^{15,21,22} Dentin or saliva can display the effects of MMPs at as low a pH as 4.5, though the MMPs cannot disrupt the dentin organic matrix at that value. However, when pH levels are normal, MMPs are activated and disrupt the demineralized collagen-rich organic matrix left on the dentin after the acid effect.^{14,23,24} A wide variety of MMPs has been identified in human dentin. It is believed that MMPs are involved in the early stage of dentinogenesis, the maturation stage, the erosion process and the calcification stages of internal and external dentinal tubules.

The aim of this study was to compare the effects of sodium fluoride, CHX and EGCG on dental erosion. The null hypothesis tested was that CHX and EGCG materials would be as effective as a fluoride solution in preventing dental erosion.

Materials and methods

Teeth selection

This study was approved by the Istanbul University, Faculty of Dentistry, Clinical Research Ethics Committee (Protocol No. 2016/27). Forty-four (44) recently extracted non-carious human third molars were used in this analysis. The sample size calculation resulted in an 80 % power at the 5 % statistical significance level and a 10 % difference between the groups. Though seven (7) samples were required

for each group, our analysis exceeded that requirement, using 10 samples for each group. Written consent was obtained from patients and they were informed as to the usage of their teeth for research purposes. Teeth were stored in 0.9 % NaCl with 0.2 % NaN₃ at 4°C until studied.

Sample preparation

The crowns were sectioned from the roots and the dentin slabs were cut (4×4 mm) with a diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). The samples were fixed on acrylic rods with a sticky wax and formed a ground flat with water-cooled carborundum discs (320, 600 and 1,200 grades of Al₂O₃ paper, Buehler). They were polished with felt paper wet by diamond spray (1 mm, Buehler) on a rotating polishing machine and cleaned in an ultrasonic cleaner (SONICA Sweep System, Soltec, Milan, Italy) for 2 minutes. The profilometry analysis reference area was established on every sample through the application of nail varnish.

Each sample was immersed in 30 mL of Coke® (330 ml, canister, pH 2.6, Coca-Cola Company, USA) for 1 minute, 4 times a day for 5 days to promote erosive demineralization. A new canister was opened for each sample and stored at room temperature for 5 minutes. Between the demineralization phases, the samples were rinsed in an ultrasound bath for 2 minutes and stored in artificial saliva containing 0.2 mM glucose, 9.9 mM NaCl, 1.5 mM CaCl₂·2H₂O, 3 mM NH₄Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mM K₂HPO₄, 3.3 mM urea, 2.4 mM NaH₂PO₄, 0.05 mM ZnCl₂ and traces of ascorbic acid (pH 6.8); freshly prepared artificial saliva was replaced daily.

Measurements

The first measurements (T1) of surface roughness were performed with the stylus profiler (KLA Tencor, P-6). Initial dental topographic characteristics (T1) were assessed using a scanning electron microscope (SEM, FEI Versa 3D Dual Beam).

After the demineralization process, the samples were randomly assigned to four groups. Each group was treated with a different material. Ten (10) of the 11 samples in each group were used for profilometric measurements and one, non-coated sample was reserved for SEM examination.

The groups (n=11)

Group 1: Sodium fluoride (1.100 ppm NaF) (positive control) (Sigma, Steinheim, Germany)

Group 2: 0.61 % EGCG (OM24, powder, Omnimedica, Zurich, Switzerland)

Group 3: 12 % CHX (Sigma, Steinheim, Germany)

Group 4: 0.2 % CHX (Sigma, Steinheim, Germany)

All solutions were prepared fresh each day according to the manufacturer's instructions. The fluoride formulation was prepared in the authors' faculty laboratory using an NaF solution and deionized water. The green tea extract solution was composed of a mixture of 6.1 mg of EGCG powder and 10 ml of deionized water.

For the remineralization process, the samples were immersed in 0.61 % EGCG, 12 % CHX, 0.2 % CHX and the positive control group solution (1.100 ppm NaF) for 1 minute, 4 times a day for 5 days. Between the remineralization phases, the samples were cleaned in an ultrasonic bath for 2 minutes and stored in artificial saliva. Changes to the dentin surface were measured according to surface roughness using a stylus profiler and SEM (T2).

Surface analysis of dentin

During analysis, the evaluation of the dentinal surface profile was performed using a stylus profiler. The points of roughness measurement were randomly marked on the sample surface. For each sample, Ra (defined as the average distance from the profile to the mean line over the length of assessment) measurements were made. The topographic characteristics were assessed by SEM with X800 magnification.

Statistics

Statistical analyses were performed using IBM SPSS Statistics 22. The Shapiro Wilks test revealed that the parameters did not show normal distribution. A Kruskal Wallis test was thus used to compare the parameters and a Mann Whitney U test was used to determine the group differences. In-group

comparisons of parameters were performed using Wilcoxon Signed Ranks. The significance level was set at $p < 0.05$.

Results

There was a significant difference between the groups in terms of T1 measurement values ($p: 0.000$). The T1 values of the EGCG group were found to be statistically significantly higher than the positive control and 0.2 % CHX groups ($p_1: 0.000; p_2: 0.000; p < 0.05$). The T1 values of the 12 % CHX group were found to be significantly higher than the positive control and 0.2 % CHX groups ($p_1: 0.000; p_2: 0.000; p < 0.05$) (Table 1; Fig. 1).

Table 1: Evaluation of T1 and T2 measurements between and within groups

Grup	T1	T2	T1-T2 p^2
	Mean±SS (medyan)	Mean±SS (medyan)	
Positive control (NaF)	0.165±0.08 (0.156)	0.165±0.08 (0.155)	0.379
EGCG	0.476±0.34 (0.362)	0.198±0.09 (0.194)	0.000*
12% CHX	0.394±0.22 (0.353)	0.295±0.23 (0.180)	0.004*
0.2% CHX	0.152±0.08 (0.146)	0.151±0.09 (0.122)	0.694
p^1	0.000*	0.000*	

¹Kruskal Wallis test

²Wilcoxon sign test

* $p < 0.05$

A statistically significant difference was also found between the groups in terms of T2 measurement values ($p: 0.000; p < 0.05$). Pairwise comparisons demonstrated that T2 values of the 0.2 % CHX group were found to be statistically significantly lower than the positive control, EGCG and 12 % CHX groups ($p_1: 0.035; p_2: 0.000; p_3: 0.000; p < 0.05$). The T2 values of the positive control group were found to be statistically significantly lower than the EGCG and 12 % CHX groups ($p_1: 0.005; p_2: 0.008; p < 0.05$). There was no statistically significant difference between the T2 measurements of the

EGCG and 12 % CHX groups ($p>0.05$), nor was there any statistically significant change in T2 measurement compared to T1 measurement in the positive control group and in the 0.2 % CHX group ($p>0.05$). The decrease in T2 measurement as compared to T1 measurement was statistically significant in the EGCG group and in the 12 % CHX group ($p: 0.000$; $p<0.05$) (Table 1; Fig. 1).

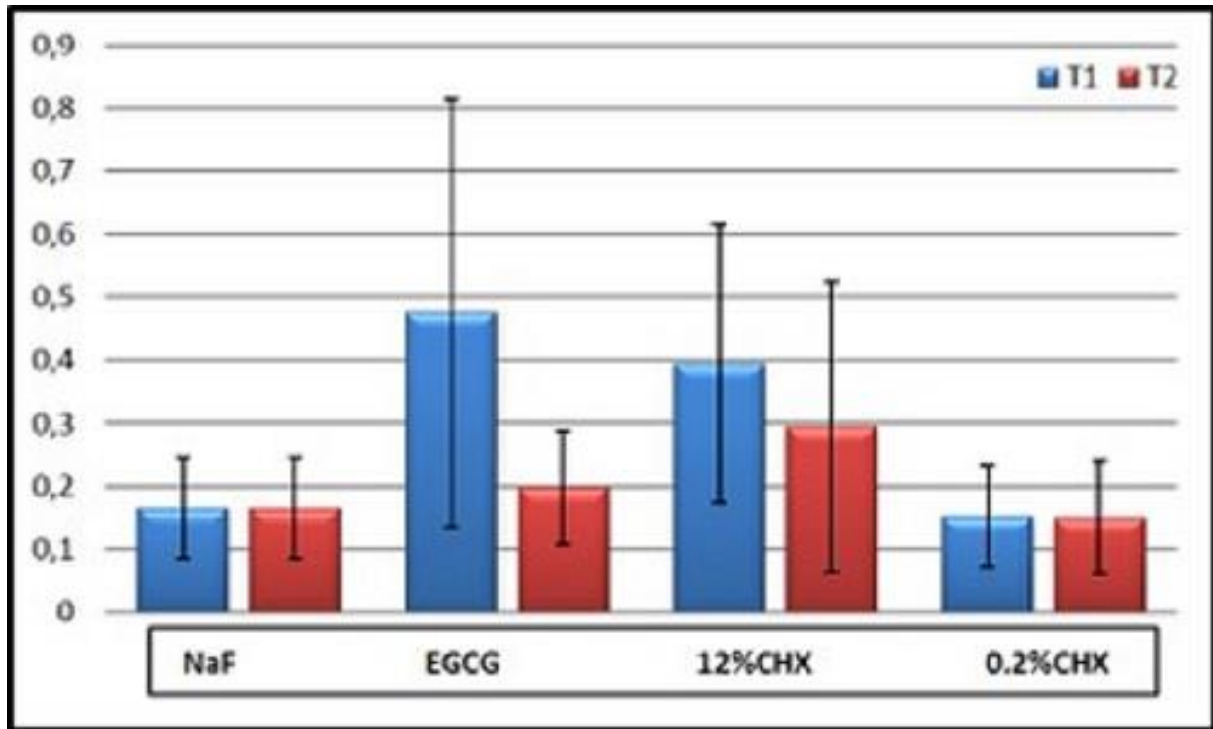


Figure 1: Graphical representation of T1-T2 measurements between groups

A statistically significant difference was found among the groups in terms of decreases in T2 measurement as compared to T1 measurement ($p: 0.000$; $p<0.05$). Pairwise comparisons showed that the decrease in the EGCG group was statistically significantly higher than the positive control, 12 % CHX and 0.2 % CHX groups ($p_1: 0.000$; $p_2: 0.011$; $p_3: 0.000$; $p<0.05$). The decrease in the 12 % CHX group was statistically higher than the positive control and 0.2 % CHX groups ($p_1: 0.004$; $p_2: 0.004$; $p<0.05$). There was no statistically significant difference between the positive control and 0.2 % CHX groups in terms of the decrease in T2 measurement as compared to T1 measurement ($p>0.05$) (Table 2; Fig. 2).

Table 2: Evaluation of the decrease in T2 measurement according to T1 measurement between groups

Group	T1-T2
	Mean±SS (medyan)
Positive control	0.0006±0.10 (0.009)
EGCG	0.278±0.35 (0.152)
12% CHX	0.115±0.31 (0.115)
0.2% CHX	0.0005±0.11 (0.006)
p¹	0.000*
<i>Kruskal Wallis test</i>	<i>*p<0.05</i>

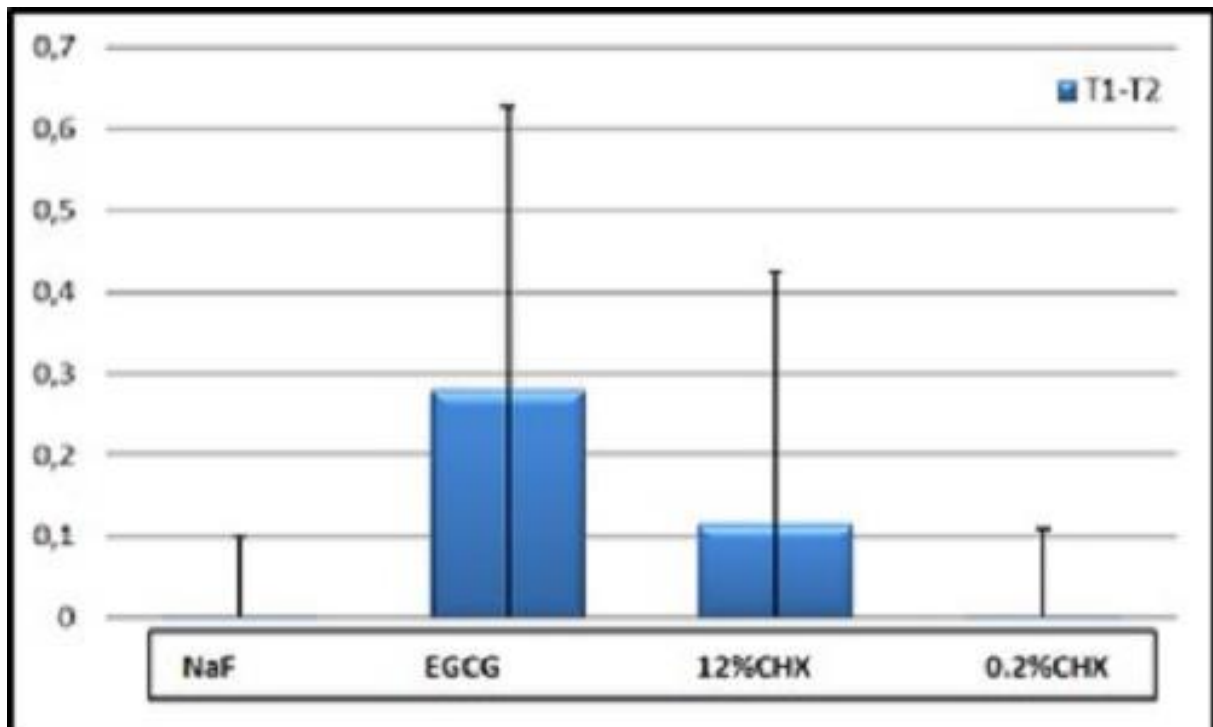


Figure 2: Graphical representation of T2-T1 reduction between groups

The initial view of all materials showed a smooth, intact dentin surface with no evidence of erosion. After demineralization, porosity zones and interprismatic dissolution were visible (T1). The decrease in porosity and interprismatic dissolution zones can be seen in the SEM images after remineralization (T2). Analysis revealed that the remineralization images of the EGCG group were observed more frequently than those of the other groups (Fig. 3).

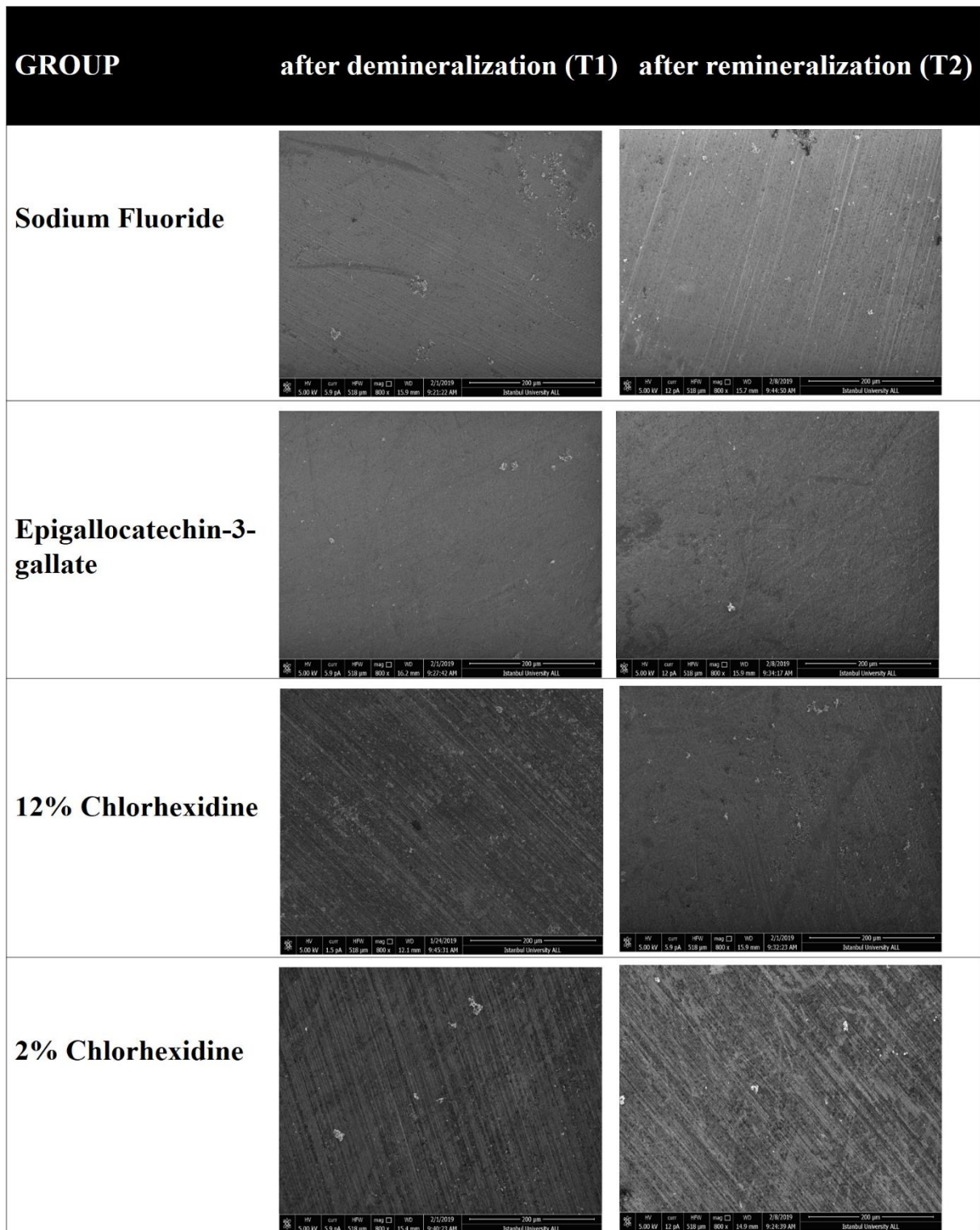


Figure 3: SEM images of the materials

Discussion

This study investigated the effect of green tea extract (EGCG) and CHX on demineralization. There exist many *in vitro* studies with varying methodologies, mostly as relates to the demineralization agents investigated and the application times. For example, when the literature was examined it was noted that some studies employed Coke®^{10,14} and others used orange juice.²⁵

Profilometry is a frequently used technique in dentistry and materials engineering. One of the most common measurement methods of enamel or dentin surface loss is stylus profilometry. Although this technique is associated with an inability to detect narrow areas and sample damage, stylus profilometry is frequently used in dental studies due to its precise qualitative and quantitative assessments of dental surface topography.^{26,27} Ra value, which refers to the arithmetic average of surface roughness, is one of the most common surface roughness parameters.^{27,28} Ra surface parameter was used in this study for comparative analysis of demineralization and remineralization.

This study followed the methodology of previous studies^{29,30} regarding the doses for NaF, CHX and EGCG. Because the MMP inhibitory effect of CHX is dose-dependent, two different doses of CHX were used.³¹

EGCG and CHX were found to reduce dental erosion significantly more than NaF. This finding is consistent with previous studies. This may be due to CHX and polyphenols being able to interact with metal ions, which could have allowed particle accumulation in tubules by binding to Ca ions. This interaction should have affected de/remineralization processes by reducing dental erosion. It has previously been demonstrated that under clinical conditions CHX and green tea extract solutions decrease dentin erosion almost as successfully as a conventional 250 ppm fluoride solution.¹⁵ The intrinsic limitation of *in vitro* studies, however, is that the demineralization and remineralization cycle is carried out under *in vitro* conditions; the results are therefore not completely transferable to an *in vivo* environment in which the oral cavity is the natural protective environment. In this study, it was attempted to replicate the oral environment insofar as possible using variables such as erosive solution and artificial saliva.

An evaluation of the effect of the inhibition of endogenous dentinal enzymes [MMPs and cysteine cathepsins (CCs)] on dentin erosion reported that in comparison to a placebo, a gel treatment of 1.23 % NaF decreased the dentin loss slightly, though at a rate without statistical significance.³² This result was interpreted to mean that different fluoride compounds might have a better protective effect against erosion than sodium fluoride. In the current study, it was concluded that EGCG and 12 % CHX were as effective as sodium fluoride.

Several previous studies have reported results similar to those of the present study as regards the role of green tea in reducing erosion.^{11,15,33} Another study concluded that a reasonable protective measure to reduce dentin erosion potential might be to include green tea extract in soft drinks.¹² In recent years several naturally derived substances have been discovered to inhibit MMP. One of the green tea polyphenols, EGCG, is a type of catechin which exhibits a wide variety of biological and pharmacological properties. Many studies have reported that cleaning the oral cavity with green tea reduces dental erosion significantly better than cleaning with water.^{15,16,34} The mechanism of the EGCG's inhibitory effect on MMPs is based on hydrogen bonding and hydrophobic interactions with the collagenase. EGCG may cause conformational change of MMP-2 and inhibit the activation of MMP-8 by affecting the remineralization of demineralized dentin.³⁴

A 2014 study examined the effects of fluoride and EGCG on *in vitro* dental erosion. Samples were analyzed using a laser scanning confocal microscope and SEM.²⁵ It was concluded that both fluoride and EGCG were effective in preventing dental erosion as compared to the control group, but did not show a combined effect when applied together.

Green tea has been reported to increase the surface microhardness of eroded dentin, the improvement being confirmed by SEM evaluation of surface appearance and obvious depositions.³⁵ According to the SEM images produced during this study, EGCG were observed more regularly than the other materials. Many studies use SEM to evaluate surface characterization as well as to observe changes in specific morphological and structural features^{8,36} and it has been suggested that treatments with MMP inhibitors may have changed the nanostructure of collagen fibrils in SEM images.³³ We therefore

evaluated the SEM images in one sample to observe structural change and found more ordered structures in EGCG applied samples.

The decline of dentin loss under mild in vitro erosive and abrasive conditions with the usage of toothpastes containing CHX or green tea extract has been demonstrated. These materials were found to be almost as efficient as conventional toothpaste containing 1.100 ppm F.³¹ In this context, it may be beneficial to include EGCG and CHX as additives to dental materials intended for individuals susceptible to erosion and abrasion.

Conclusion

The null hypothesis of the present study that the 12 % CHX and 0.61 % EGCG solutions have been shown to be as effective as fluoride solutions has been verified. We think that these materials can be an alternative approach to the use of fluoride in remineralization. In particular, the use of green tea polyphenols in every field continues to be investigated, further in vivo and in vitro studies are required to confirm the inhibitory effect of these solutions on MMPs.

Competing Interests

The authors declare that there is no competing interest

References

1. Chan AS, Tran TTK, Hsu YH, Liu SYS, Kroon J. A systematic review of dietary acids and habits on dental erosion in adolescents. *Int J Paediatr Dent.* 2020;30:713–33.
2. Asher C, Read MJF. Early enamel erosion in children associated with the excessive consumption of citric acid. *Br Dent J.* 1987;162:384–87.
3. Benjakul P, Chuenarrom C. Association of dental enamel loss with the pH and titratable acidity of beverages. *J Dent Sci.* 2011;6:129–33.
4. Lussi A, Buzalaf MAR, Duangthip D, Anttonen V, Ganss C, João-Souza SH, Carvalho TS. The use of fluoride for the prevention of dental erosion and erosive tooth wear in children and adolescents. *Eur Arch Paediatr Dent.* 2019;20:517–27.
5. Almeida de Silva JS, Baratieri LN, Araujo E, Widmer N. Dental erosion: understanding this pervasive condition. *J Esthet Restor Dent.* 2011;23:205–16.
6. Amoras DR, Corona SA, Rodrigues Jr AL, Serra MC. Effect of beverages on bovine dental enamel subjected to erosive challenge with hydrochloric acid. *Braz Dent J.* 2012;23:367–72.
7. Johansson AK, Omar R, Carlsson GE, Johansson A. Dental erosion and its growing importance in clinical practice: from past to present. *Int J Dent.* 2012;2012:632907.
8. Wang YL, Chang HH, Chiang YC, Lu YC, Lin CP. Effects of fluoride and epigallocatechin gallate on soft-drink-induced dental erosion of enamel and root dentin. *J Formos Med Assoc.* 2018;117:276-82.
9. Cvikl B, Lussi A, Carvalho TS, Moritz A, Gruber R. Stannous chloride and stannous fluoride are inhibitors of matrix metalloproteinases. *J Dent.* 2018;78:51–8.
10. Lussi A, Schlueter N, Rakhmatullina E, Ganss C. Dental erosion—an overview with emphasis on chemical and histopathological aspects. *Caries Res.* 2011;45:2–12.
11. De Moraes MDR, Carneiro JRM, Passos VF, Santiago SL. Effect of green tea as a protective measure against dental erosion in coronary dentine. *Braz Oral Res.* 2016;30:1–6.

12. Barbosa CS, Kato MT, Buzalaf MA. Effect of supplementation of soft drinks with green tea extract on their erosive potential against dentine. *Aust Dent J.* 2011;56:317–21.
13. Buzalaf MAR, Magalhães AC, Wiegand A. Alternatives to fluoride in the prevention and treatment of dental erosion. *Monogr Oral Sci.* 2014;25:244–52.
14. Buzalaf MAR, Kato MT, Hannas AR. The role of matrix metalloproteinases in dental erosion. *Adv Dent Res.* 2012;24:72–6.
15. Magalhães AC, Wiegand A, Rios D, Hannas A, Attin T, Buzalaf MA. Chlorhexidine and green tea extract reduce dentin erosion and abrasion in situ. *J Dent.* 2009;37:994–8.
16. Kato MT, Magalhães AC, Rios D, Hannas AR, Attin T, Buzalaf MAR. Protective effect of green tea on dentin erosion and abrasion. *J Appl Oral Sci.* 2009;17:560–4.
17. Maleki A, Daraei H, Mohammadi E, Zandi S, Teymouri P, Mahvi AH, Gharibi F. Daily Fluoride Intake from Iranian Green Tea: Evaluation of Various Flavorings on Fluoride Release. *Environmental Health Insights* 2016;10:59-63.
18. Scheutzel P. Etiology of dental erosion—intrinsic factors. *Eur J Oral Sci.* 1996;104:178–90.
19. Frazao JB, Machado LG, Ferreira MC. Dental erosion in schoolchildren and associated factors: a cross-sectional study. *J Indian Soc Pedod Prev Dent.* 2018;36:113–9.
20. Corica A, Caprioglio A. Meta-analysis of the prevalence of tooth wear in primary dentition. *Eur J Paediatr Dent.* 2014;15:385–8.
21. Hara AT, Ando M, Cury JA, Serra MC, González-Cabezas C. Influence of the organic matrix on root dentine erosion by citric acid. *Caries Res.* 2005;39:134–8.
22. Kato MT, Leite AL, Hannas AR, Calabria MP, Magalhães AC, Pereira JC, Buzalaf MAR. Impact of protease inhibitors on dentin matrix degradation by collagenase. *J Dent Res.* 2012;91:1119–23.
23. Kreulen CM, Van't Spijker A, Rodriguez JM, Bronkhorst EM, Creugers NH, Bartlett DW. Systematic review of the prevalence of tooth wear in children and adolescents. *Caries Res.* 2010;44:151–9.

24. Lussi A, Hellwig E, Zero D, Jaeggi T. Erosive tooth wear: diagnosis, risk factors and prevention. *Am J Dent.* 2006;19:319–25.
25. Wang YL, Chang CC, Chi CW, Chang HH, Chiang YC, Chuang YC, Chang HH, Huang GF, Liao YS, Lin CP. Erosive potential of soft drinks on human enamel: an in vitro study. *J Formos Med Assoc.* 2014;113:850–6.
26. Kato MT, Leite AL, Hannas AR, Oliveira RC, Pereira JC, Tjäderhane L, Buzalaf MAR. Effect of iron on matrix metalloproteinase inhibition and on the prevention of dentine erosion. *Caries Res.* 2010;44:309–16.
27. Barac R, Gasic J, Trutic N, Sunaric S, Popovic J, Djekic P, Radenkovic G, Mitic A. Erosive effect of different soft drinks on enamel surface in vitro: application of stylus profilometry, *Med Princ Pract.* 2015;24:451–7.
28. Field J, Waterhouse P, German M. Quantifying and qualifying surface changes on dental hard tissues in vitro. *J Dent.* 2010;38:182–90.
29. Alexandria AK, Vieira TI, Pithon MM, Da Silva Fidalgo TK, Fonseca-Gonçalves A, Valença AMG, Cabral LM, Maia LC. In vitro enamel erosion and abrasion-inhibiting effect of different fluoride varnishes. *Arch Oral Biol.* 2017;77:39–43.
30. Ren YF, Zhao Q, Malmstrom H, Barnes V, Xu T. Assessing fluoride treatment and resistance of dental enamel to soft drink erosion in vitro: applications of focus variation 3D scanning microscopy and stylus profilometry, *J Dent.* 2009;37:167–76.
31. Hannas AR, Kato MT, Cardoso CAB, Magalhães AC, Pereira JC, Tjäderhane L, Buzalaf MAR. Preventive effect of toothpastes with MMP inhibitors on human dentine erosion and abrasion in vitro. *J Appl Oral Sci.* 2016;24:61–6.
32. Zarella BL, Cardoso CAB, Pelá VT, Kato MT, Tjäderhane L, Buzalaf MAR. The role of matrix metalloproteinases and cysteine-cathepsins on the progression of dentine erosion. *Arch Oral Biol.* 2015;60:1340–5.
33. Kato MT, Leite AL, Hannas AR, Buzalaf MAR. Gels containing MMP inhibitors prevent dental erosion in situ. *J Dent Res.* 2010;89:468–72.

34. Magalhães AC, Wiegand A, Rios D, Buzalaf MAR, Lussi A. Fluoride in dental erosion. *Monogr Oral Sci.* 2011;22:158–70.
35. Mirkarimi M, Toomarian L. Effect of green tea extract on the treatment of dentin erosion: an in vitro study. *J Dent (Tehran).* 2012;9:224–8.
36. Zhang XZ, Anderson P, Dowker SEP, Elliott JC. Optical profilometric study of changes in surface roughness of enamel during in vitro demineralization, *Caries Res.* 2000;34:164–74.

Tables and Figure Legends

Table 1: Evaluation of T1 and T2 measurements between and within groups

Table 2: Evaluation of the decrease in T2 measurement according to T1 measurement between groups

Figure 1: Graphical representation of T1-T2 measurements between groups

Figure 2: Graphical representation of T2-T1 reduction between groups

Figure 3: SEM images of the materials