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Protection against Dental Erosion and the Remineralization Capacity of Non-Fluoride Toothpaste, Fluoride Toothpaste and Fluoride Varnish

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Abstract: Introduction: The oral cavity and the teeth are frequently subjected to numerous physiological pH variations, mainly due to the type of diet. These changes are the main cause of enamel demineralization and consequent breakage under mechanical forces. This causes severe sensitivity and sometimes pain, which endures unless the hard tissue is remineralized. Since enamel does not self-repair, the application of alloplastic materials that have the property of releasing remineralizing ions is strongly recommended. The aim of this in vitro study is to evaluate the effectiveness of two different toothpastes and a fluoride varnish in the prevention of demineralization, and their ability to remineralize enamel after it has undergone several acidifications. Methods: Eight human teeth with no caries or defects were prepared. The acid attack simulations were performed using a commercial cola carbonated drink. Samples were immersed in 5 mL of soft drink for two minutes at room temperature, and then were rinsed with distilled/deionized water. The immersion process lasted about 2 min, and was repeated four times for a total of eight minutes. Then, two different types of toothpaste, one with and one without fluoride, and a fluoride varnish were applied to the surfaces of the samples and rinsed off with demineralized water. A second acidification cycle was then carried out, with the subsequent reapplication of the three different products to evaluate their ability to protect against demineralization. SEM and profilometer analysis were then carried out to evaluate the results. Results: The statistical analysis showed a good remineralization capacity for all three products, especially in the fluoride-varnish-treated samples. However, regarding protection from demineralization, non-fluoride toothpaste was found to be ineffective, while fluoride toothpaste and varnish produced positive results. Conclusions: Dental professionals should advise fluoride toothpastes and varnishes over non-fluoride toothpastes. Fluoride toothpastes and varnishes represent a valid treatment for surface remineralization after a first acid attack and for protection from subsequent demineralization in an acid environment, such as that which can develop in the oral cavity.

Keywords: dental erosion; remineralization; toothpaste; fluoride; varnish

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1. Introduction

Dental erosion is considered a partial chemical dissolution of the hard tissues of the tooth surface. This phenomenon is caused mainly by repeated exposure to acids of both microbiological and non-microbiological origin. Simultaneous and/or subsequent exposure to mechanical forces might result in tooth surface loss [1]. Furthermore, the dissolution of enamel is closely associated with some precise chemical parameters, such as pH, saliva buffering capacity, and quantity, and external factors, such as acidity, viscosity, calcium, phosphate and fluoride ion concentration in drinks and food [2]. It is widely demonstrated that these parameters have a direct influence on tooth surface strength, and this is the reason why they are normally used to calculate the degree of saturation of various substances, so as to quantify the demineralizing force in hard tissues [3].

Substances with a low pH, high titratable capacity and high buffering capacity have the greatest erosive force; conversely, substances with high concentrations of the Ca^{2+} ion and phosphate cause the least dissolution of the tooth surface, and can enhance its chemical stability and integrity [4].

At the present time, dental erosion is a daily problem, accentuated by the consumption of acidic drinks, which have a high demineralizing potential. Unfortunately, a lot of chemicals, additives and sugar are hidden in most food and drinks of regular intake, such as carbonated, alcoholic, non-alcoholic and sports drinks and fruit juices. These substances have the capability to decrease the pH of the saliva, which enhances the ongoing process of dental erosion [5]. As far as the origin of the acid is concerned, extrinsic and intrinsic erosions can be distinguished. In fact, the extrinsic ones are generally caused by acids in food, and the severity of the erosion is associated with several factors, such as nutrition, saliva and mechanical and frictional stress [6]. An important factor is contributed by the digestive enzymes released by the salivary glands, and, in particular, by their response to hunger and various food nutrients. In fact, these enzymes were found to increase their activity in response to food, revealing the dependence of their production on food [7].

It is known that tooth enamel is made up mostly of inorganic material, which represents about 96%, while the remaining 4% is organic material and water [8]. The inorganic material consists of hydroxyapatite crystals, which comprise a mineral with the chemical composition $Ca_{10}(PO_4)_6(OH)_2$ with a hexagonal prismatic structure. Enamel, which is predominantly inorganic, is not composed of living cells with specific repair capabilities with regard to diseases such as caries, abrasions and/or fractures [9]. When this happens, erosive tooth wear might occur, especially in the cervical region, just above the gingival margin, on the incisal edge of anterior teeth or on the cusps of posterior teeth. It is in particular the mechanical forces imposed on anterior teeth that can ultimately lead to the eventual breakage of a tooth in the esthetic region. A similar situation could cause an impairment, not only from an esthetic point of view, but also in terms of the masticatory function, and speech and labial support might be highly compromised. In the case of a growing child, changes in the occlusal vertical dimension may lead to an incorrect development of bones, muscles and temporomandibular joints [10,11].

To sum up, when an acidic environment is formed inside the oral cavity and the surfaces of the enamel are consequently exposed, a process of demineralization begins, which may vary from person to person. To slow down this process, the use of specific products, such as toothpastes and fluoride varnish, allows repair by remineralization [12]. Moreover, the remineralization process can be also induced by products such as glass ionomer cements, which have the ability to release fluorine ions and composite materials that can release $CaPO_4$ anions. These materials have the capability to induce apatite formation through the presence of bioactive glass and they are able to absorb fluoride ions from toothpastes and fluoride varnishes and subsequently release them in the oral cavity [13,14]. Through them, the enamel can be protected and enhanced in resistance to acid attacks; therefore, these products offer protection against the erosion of the enamel when they contain certain fluoride concentrations [15]. The hydroxyapatite is dissolved in acidic environment. However, if fluoride ions are available in the oral environment, this element can form with HA-fluorohydroxyapatite. The fluoride is absorbed by the crystals on the surface, thus attracting calcium ions. Apatite without carbonates is less soluble, so in the case of demineralization it replaces the original mineral [16]. The new layer is characterized by a lower solubility due to the replacement of carbonate with fluoride, making the surface of the enamel less subject to acid erosion. Fluoridated toothpaste, gel enriched with fluoride and fluoride varnishes still represent the best options available on the market: they are easy to obtain and even easier to use or apply. Gel and toothpaste are widely recommended by pediatric dentists, and they can even be used at home on a daily basis. Apart from these materials, several others have been developed in the last few years: nowadays, conventional flowable and high-strength universal injectable composites represent a concrete solution for the pediatric population for both deciduous and permanent dentition. The importance of these restorative materials is their capability to release fluoride and other compounds that improve hard tissue remineralization [17,18]. In fact, current research aims to identify various elements that make dental tissues harder and more effective in contrasting demineralization [19].

The Aim of the Study

The purpose of this study is to investigate the effectiveness of two different types of toothpaste (one with fluoride and one without) and a fluoride varnish in preventing erosion caused by an acidic environment present in the oral cavity and to protect hard tissues from acids.

The first null hypothesis is that, as far as erosion prevention is concerned, there is no difference between the two toothpastes and the fluoride varnish.

The second null hypothesis is that, as far as protection from a second acid attack is concerned, there is no difference between different toothpastes and the fluoride varnish.

2. Materials and Methods

2.1. Sample Preparation

Eight healthy human teeth with no caries and/or defects were extracted for periodontal reasons. Ethical approval was waived by the ethic committee. Following the extraction, the soft tissue residues were removed from the teeth, which were then analyzed to find any fractures. Fracture analysis was conducted with an optical microscope (Leica Microsystems DM300, Germany) using 4X, 10X, 40X and 100X magnification. Then, the teeth were disinfected in sodium hypochlorite titrated at 5% in active chlorine for 1 h, and were stored in a 0.9% NaCl solution containing 0.1% thymol throughout the treatment so that they remained hydrated. The samples were cut with a high-speed diamond bur (KG®) and water irrigation at the level of the enamel–dentin junction, and then sectioned, so that the flat surface was opposite to the treated one. To make the surfaces uniform, the samples were polished with silicon abrasive tips [5]. According to the adopted procedure, samples were divided into 8 groups, which are illustrated below in Table 1. Table 2 shows the product composition.

Table 1. Groups divided by surface treatment.

Groups	Procedure
G 1	Rubber polisher
G 2	Rubber polisher + soft drink
G 3	Rubber polisher + soft drink + non-fluoride toothpaste
G 4	Rubber polisher + soft drink + fluoride toothpaste
G 5	Rubber polisher + soft drink + fluoride varnish
G 6	Rubber polisher + soft drink + non-fluoride toothpaste + soft drink

G 7	Rubber polisher + soft drink + fluoride toothpaste + soft drink
G 8	Rubber polisher + soft drink + fluoride varnish + soft drink

Table 2. The composition of the products used for the process.

Product	Composition
Non-fluoride toothpaste	Aloe vera, chamomile, echinacea, horse chestnut, mint, carboxymethylchitosan, hydrated silica and xylitol
Fluoride toothpaste	Calcium carbonate, water, sorbitol, aroma*, poloxamer 407, sodium monofluorophosphate, cocamidopropyl betaine, zinc oxide, benzyl alcohol, cellulose gum, zinc citrate, sodium bicarbonate, tetrasodium pyrophosphate, xanthan gum, sodium saccharin, sucralose, limonene, CI 77891
Fluoride varnish	Active ingredient: sodium fluoride: 1 mL = 22.6 mg of fluoride excipients: 96% ethanol, white wax (E901); shellac (E904), rosin, mastic, saccharin (E954), raspberry essence

2.2. *Demineralization and Remineralization*

In order to simulate the demineralization process, a carbonated soft drink (CocaCola®, Milano, Italy) was chosen. The pH at 20 °C, buffering capacity, concentration of calcium and phosphate of the drink were measured. Measurements were performed in triplicate (5).

Samples G2, G3, G4, G5, G6, G7 and G8 were immersed in 5 mL of soft drink for two minutes at room temperature, and then they were rinsed with distilled/deionized water.

The immersion process lasted about 2 min and was repeated four times for a total of eight minutes. The three different products were applied, without brushing, to cover the entire surface of the enamel of some samples, and then they were rinsed with distilled water.

Sample G1 did not have any chemical changes; the only treatment was polishing with silicon abrasive tips (3M®). No products were applied to sample G2; the only procedure was the initial acidification. The different products were applied in the same way: they were placed in contact with the enamel surface for three minutes at zero hour, and again after 8, 24 and 36 h. (5)

Samples "3" and "6" were treated with a non-fluoride toothpaste; samples "4" and "7" were treated with a 1450 ppm of fluoride toothpaste. Finally, samples "5" and "8" were treated with single 0.5 mL doses of professional fluoride varnish specific for dental hypersensitivity and containing 22,600 ppm of fluoride. Samples "6", "7" and "8" were subjected to another acidification cycle for the evaluation of the effectiveness protection of the products following a second acid attack.

2.3. *Scanning Electron Microscope (SEM)*

The morphological characteristics of the samples were studied using an SEM (Leica Microsystems srl, Milan, Italy). In order to conduct a proper observation, conductive samples were required, so a metal layer was firstly placed on top of them. In detail, the samples of this study were coated with a gold layer of approximately 20 nm. The microscope used for the experimental activity was a Cambridge Stereoscan 440 (Leica Microsystems srl, Milan, Italy) equipped with a Philips PV9800 EDS microanalysis (Leica Microsystems srl, Milan, Italy), available at the Industrial Engineering Department of the University of Padua. The images were taken using the secondary electron detector [17].

2.4. Profilometer

The surface topography of the teeth was analyzed using a Sensofar Plu Neox optical profilometer (Barcelona, Spain). Scans with lateral dimensions of 1.3 × 0.6 mm² were acquired using a 20× confocal objective. After the acquisition, the topographies were subjected to the removal of the shape by subtraction of a plan. Three scans were performed in different areas for each tooth, and three surface profiles were extracted for each topography, from which the roughness was calculated according to ISO 4288 [20]. Two filters, λ and λc, equal to 2.5 µm and 0.025 mm, were applied for the roughness calculation [18].

3. Results

Five measurements were made for each sample, but in different locations. Please check Table 3 for further clarification.

Table 3. Profilometer measurements.

	Ra1	Ra2	Ra3	Ra4	Ra5	Media	Standard Deviation
1	0.4778	0.4683	0.4508	0.4375	0.513	0.46948	0.02888022
2	1.9845	2.0159	1.9518	2.3981	2.5405	2.17816	0.27144762
3	0.9504	0.8388	0.8195	1.0379	1.0858	0.94648	0.11779426
4	0.9042	1.1652	1.0916	0.9886	0.9196	1.01384	0.11235617
5	0.9337	0.6827	0.5569	0.565	0.5016	0.64798	0.17280751
6	1.118	0.9084	0.8817	1.0521	1.3365	1.05934	0.18347338
7	0.7099	0.7319	0.8403	0.8248	1.002	0.82178	0.11557131
8	1.5126	1.7345	1.4272	0.9311	1.0254	1.32616	0.33846695

Statistical analysis was carried out using R Statistical Software. Figure 1 shows the boxplot of the eight treatments considered in this study. It is possible to observe that the G2 group differed from the other groups with regard to the roughness, having the highest value. The group with the second highest roughness was G8. Among all groups, G2 and G8 exhibited greater variability than the others. Regarding G1, it can be noted that it had the lowest values, while G5 was the only group that had an outlier point. The other groups had relatively similar behaviors.

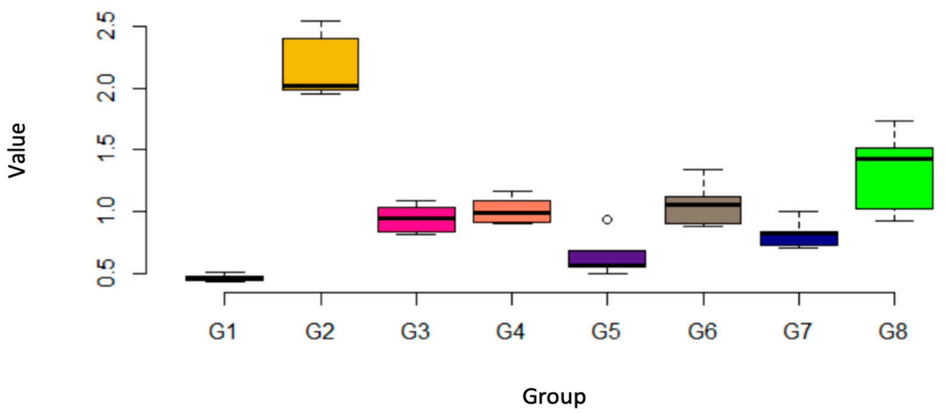

Figure 1. Boxplot of the treatments considered in the study.

In Table 4, the results with the p-value of each test are reported. It may be underlined that the values highlighted in red represent the groups that had a statistically significant difference, considering the p-value of $\alpha = 0{,}10$.

Therefore, it can be concluded, for example, that the G1 treatment presented a significant difference compared to the G2, G6 and G8 groups. In particular, G1 had on average a lower roughness value than groups "2", "6" and "8"; however, there was no statistically significant difference between group "1" and groups "3", "4", "5" and "7".

Moreover, G2 was statistically different when groups "5" and "7" were compared, showing on average a higher value than groups "5" and "7".

Finally, as far as the *p*-values between the other groups are concerned, it can be noted that these values were greater than 0.10, so the null hypothesis is not rejected, since there was no significant difference in the roughness found in the tooth.

The SEM images show the differences between the surfaces, even at higher magnification levels (Figure 2).

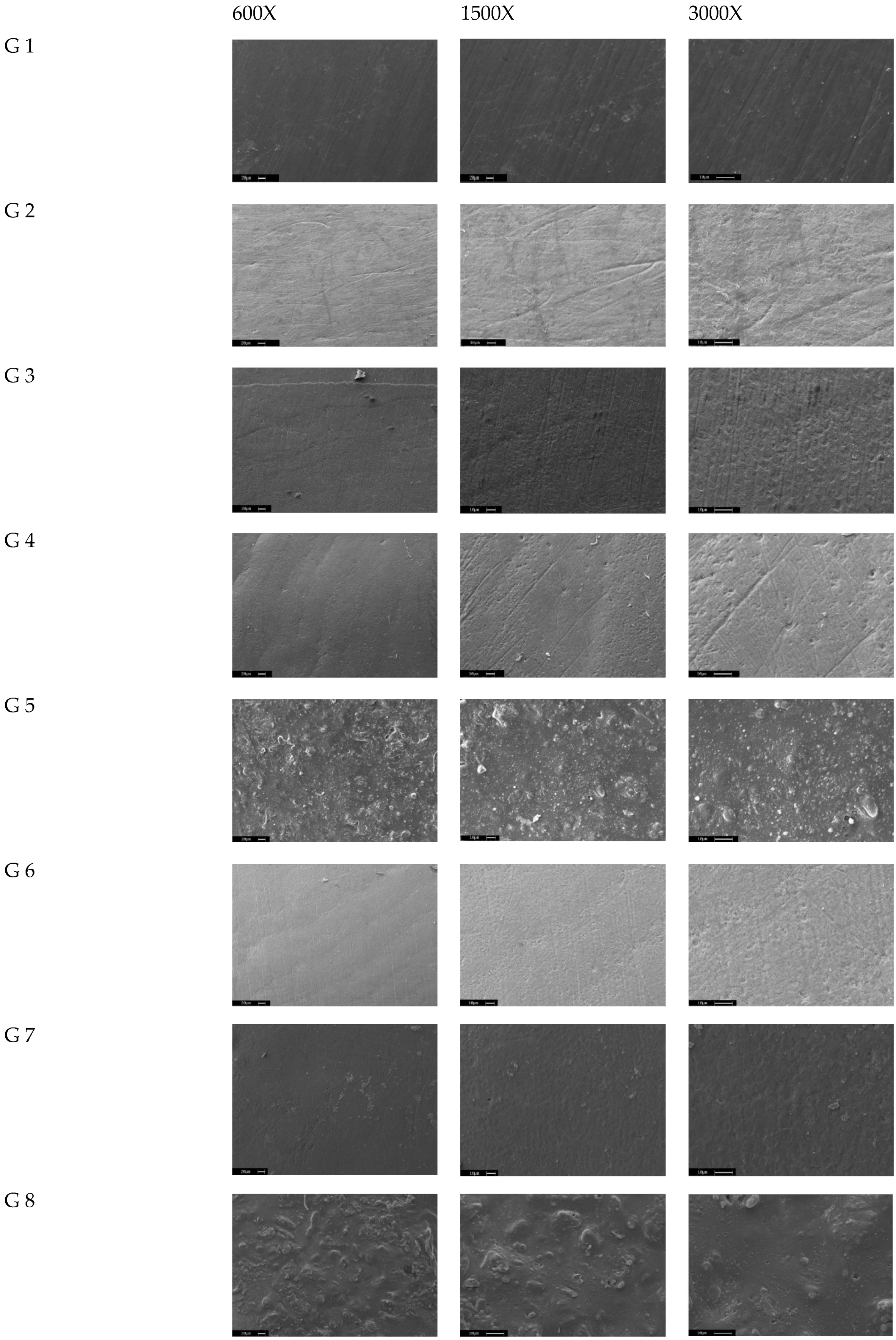
600X
1500X
3000X
G 1
G 2
G 3
G 4
G 5
G 6
G 7
G 8

Figure 2. SEM observations divided by treatment group and magnification level—600X, 1500X and 3000X.

The first null hypothesis is therefore confirmed, as no statistically significant difference between the two toothpastes and the fluoride varnish in terms of erosion prevention was reported.

The second null hypothesis is rejected, as in terms of protection from a second acid attack, the fluoride toothpaste and the fluoride varnish seemed to be more effective than the non-fluoride toothpaste

Table 4. *p*-value following the Dunnet *t*-test.

	G1	G2	G3	G4	G5	G6	G7
G2	0.0001						
G3	0.4000	0.3632					
G4	0.1227	0.9314	1.0000				
G5	1.0000	0.0041	1.0000	0.9916			
G6	0.0912	1.0000	1.0000	0.9138	0.8695		
G7	1.0000	0.0388	1.0000	1.0000	1.0000	1.0000	
G8	0.0103	1.0000	1.0000	1.0000	0.1913	1.0000	0.8613

4. Discussion

In the present study, a scanning electron microscope (SEM) and profilometer were used to test the effectiveness of two different types of toothpaste and a fluoride varnish in preventing erosion caused by an acidic environment present in the oral cavity. The SEM image results were in accordance with the findings of our study, showing differences between the surfaces even at higher magnification levels (Figure 2)

The SEM results were not sufficient to demonstrate the actual change in the enamel surfaces caused by the acid attack; a profilometer was also used for this reason. Data obtained from the roughness analysis showed that the effect of acidification was detectable by the increase in roughness. The values were G1 0.46948 μm < G2 2.17816μm. In accordance with our findings, Mullan et al., 2017, after the acidification of twenty portions of human dental enamel with of orange juice at pH 3.2, found a statistical increase in the roughness of the samples subjected to acidification, showing that soft drinks are capable of increasing enamel surface roughness [21]. Similar results were also reported by Rochel et al., 2011, where the specimens were treated with a regular cola drink at pH 2.3: their surfaces were found to have a noticeable roughness caused by acid compounds presents in the soft drink [22]. To oppose this effect, it is recommended to brush with a fluoridated toothpaste containing between 1000 ppm and 1500 ppm of fluoride. After a sugar-rich or an acid-rich meal, the pH level decreases during the first 40 min, and the risk of the enamel surface degeneration under acid attack is higher; this happens especially if teeth are brushed soon after food or drinks consumption. This phenomenon is described in the Stephen's Curve diagram, which highlights the trend of acid changes during the first 40 min after eating. For this reason, it is recommended to wait at least 20 min before brushing, and generally 2 to 3 min brushing are enough to allow the spread of fluoride and remineralizing compounds all around the surfaces of the teeth. The combination of the mechanical plaque removal and the remineralizing action of the toothpaste allows the hard tooth tissue to return to its initial surface state: shiny and smooth. In such a manner, the tooth surface is protected from demineralization, erosion and the appearance of carious lesions [23,24].

In our study, the SEM images and profilometer results demonstrate how the three different products (non-fluoride toothpaste, fluoride toothpaste and fluoride varnish) applied to G3, G4 and G5 had remineralization effects as they transformed the enamel surface to a statistically similar condition to G1 (Table 3). In support of the obtained results, the study of Olivan et al., 2020, evaluates the effect of four different products (a fluoride varnish, two different fluoride toothpastes and a non-fluoride toothpaste) on erosion lesions caused by a carbonated drink [25]. Through laser imaging of the speckle, the authors demonstrated that all the products proved able to protect the enamel from erosion. These conclusions are in agreement with the lack of statistical difference obtained by comparing the samples G1, G3, G4 and G5 in our study. These results show the importance of the use of a toothpaste or a varnish to restore the original tooth surface roughness, which, in the long term, can help in preventing more serious lesions, such as caries, teeth erosion and sensitivity.

Analyzing the SEM results, it is easily notable that the G2 surface showed a rougher surface, especially at 3000 magnification, and the enamel surface appeared more uneven, with visible furrows. In contrast with that, images of G3 and G4 at the same magnification showed the typical aprismatic appearance of an intact surface that is remineralizing. The SEM analysis was confirmed by statistical analysis, where both toothpastes, the fluoride and the non-fluoride, had the ability to restore the surfaces of G3 and G4 to a level of roughness statistically similar to the non-acidified G1. Instead, after the second acid attack in the G6 sample, there was a statistical similarity between its roughness and that of the G2 sample, and a statistical difference between its roughness and that of G1 sample, which demonstrated the inability of the non-fluoride toothpaste to protect the enamel from a second acid attack. SEM analysis corroborated the numerical results, as in the G6 images, a rougher surface could be appreciated, as well as the presence of furrows, comparable with the ones found in G2 images. These findings are in accordance with recent literature, which embraces the hypothesis that a dose-dependent effect exists between toothpaste fluoride concentration and the percentage of demineralization due to an acid oral environment [22,26]. Moreover, the G7 sample, treated with fluoride toothpaste and subjected to the second acidification cycle, shows a statistical difference compared to the G2 sample and a statistical similarity with the G1 sample. This result, when compared with the lack of statistical difference between the G2 and G6 samples, defines a better efficacy by the fluoride toothpaste in preventing enamel erosion when teeth are subjected to a second acid attack.

Looking at the literature, other studies reported results comparable to our findings: all the surfaces previously treated with a fluoridated toothpaste proved to be more resistant to enamel stress, such as tooth wear and erosion. In addition to these findings, Rochel et al., 2011, found that the synergy between xylitol and fluoride significantly reduced enamel erosion. In this study, in fact, 10% xylitol- and fluoride-enriched toothpastes proved to have a wider remineralizing action on the enamel surface compared to placebo or common toothpastes filled with xylitol or fluoride [22].

Our results are also analogous to and confirmed by the study of Gavic et al., 2018, in which 112 teeth without caries were demineralized and then treated with toothpastes containing different amounts of fluoride. The results obtained showed a tendency for micro-hardness (used in this study to measure in vitro remineralization) to increase after treatment with toothpaste containing a higher fluoride amount. Instead, the greatest decrease in micro-hardness was found in the enamel surfaces treated with non-fluoride toothpaste [27]. These results agree with the lack of statistical difference between the surface roughness of the G2 and G6 samples and with the presence of a statistical difference between the G2 and G7 samples found in our study.

The Dunnet test showed a significant statistical difference between the G1 and G8 samples. Although it initially seemed that the fluoride varnish did not provide sufficient protection of the enamel against acid attack, the G5 and G8 samples were statistically similar, which means that after the second acid attack, the roughness remained unchanged. This indicates that the G5 and G8 samples treated with fluoride varnish did not undergo variations in roughness. Therefore, this result highlights the ineffectiveness of the second acid attack. Following the instructions regarding the method of application of the fluoride varnish, an excess was created on the surfaces of the samples (as can be seen from the images taken by the SEM scanning microscope) (Figure 2). These data are interpreted as measurements of the roughness of the fluoride varnish and not of the enamel surfaces of the samples. In support of the results we obtained, Murakami et al., 2009, evaluated the effect of a cola-based drink on the surface of human enamel treated with fluoride varnish. The authors concluded that the fluoride varnish was able to inhibit erosive enamel loss [28].

Similarly, Tumbulaci et al., 2020, found that fluoride toothpaste, fluoride varnish and fluoride-enriched fissure sealant promoted hard tissue remineralization in teeth. These substances are also capable of perpetuating their action, even under an acidic environment. By precipitating calcium and phosphate into demineralized pores from saliva or external sources, remineralization therapies attempt to regain lost mineral content. In this study, fluoride varnish showed a statistically significant difference compared to placebo- and phosphate-treated surfaces. Instead, no statistically significant differences were reported between fluoride application, fluoride varnish and fluoride-enriched fissure sealant [29].

Likewise, Alexandria et al., 2017, carried on an investigation in bovine enamel specimens previously treated with soft drinks and pediatric liquid medicine. After examining 3D and SEM images, it was reported that the application of products containing CPP-ACP and NaF varnish seemed to be a promising treatment for reducing enamel loss from erosion produced by soft drinks. In this study, both varnishes also showed a great capacity to reduce tooth structure loss and surface roughness after erosion by soft drinks. In this specific case, soft drinks were combined with pediatric liquid medicine: in fact, these medications, in order to be more appealing for children, generally contain large amounts of hidden sugar [30].

Beyond everything, it must be taken into account that the density, the amount of fluoride and the method of application of fluoride varnish are different compared to toothpastes. This means that, after application, varnishes remain more consistently on the tooth surface, thus allowing a higher level of protection as well as absorption of fluoride. Therefore, depending on the level of demineralization of the tooth, it could be more convenient to combine the application of fluoride varnish every 3 to 6 months with continuous toothpaste use at home. Eventually, the prescription of gel rich in fluoride might be a good option for those children with severe early childhood caries [31].

In our study, after comparing the surface roughness of the samples treated with only the first acidification cycle and remineralized with the different toothpastes (i.e., samples G3, G4, G5), it was possible to note a statistical difference between sample G5 and G2, but not between G3 and G2 or between G4 and G2, meaning that the G5 sample seemed to be the most similar to the G1 sample. This indicates that, despite the acidification cycle, the fluoride varnish was able to repair the roughness of the enamel surface of the G5 sample to a level statistically similar to G1. Kim et al., 2018, conducted a similar study on the effectiveness of direct and indirect remineralization by fluoride varnish on artificial carious lesions. They evaluated the acid resistance of lesions remineralized by fluoride varnish and artificial saliva [32]. The results of this study corroborate our findings, confirming the remineralization effect of the fluoride varnish.

5. Conclusions

All three applied products showed a good remineralization effect on the enamel surface after the first acid attack. However, regarding protection from a second acid attack, no-fluoride toothpaste proved to be ineffective, while fluoride toothpaste produced positive results. The fluoride varnish seemed to have the ability to both remineralize and protect against acid attacks.

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References

1. González-Aragón Pineda ÁE, Borges-Yáñez SA, Irigoyen-Camacho ME, Lussi A. Relationship between erosive tooth wear and beverage consumption among a group of schoolchildren in Mexico City. Clin Oral Investig. 2019 Feb;23(2):715-723. doi: 10.1007/s00784-018-2489-8. Epub 2018 May 13. PMID: 29756172.
2. Carvalho TS, Colon P, Ganss C, Huysmans MC, Lussi A, Schlueter N, Schmalz G, Shellis RP, Tveit AB, Wiegand A. Consensus report of the European Federation of Conservative Dentistry: erosive tooth wear--diagnosis and management. Clin Oral Investig. 2015 Sep;19(7):1557-61. doi: 10.1007/s00784-015-1511-7. Epub 2015 Jul 1. PMID: 26121968.
3. Lussi A, Jaeggi T. Chemical factors. Monogr Oral Sci. 2006;20:77-87. doi: 10.1159/000093353. PMID: 16687886.
4. Saads Carvalho T, Lussi A. Chapter 9: Acidic Beverages and Foods Associated with Dental Erosion and Erosive Tooth Wear. Monogr Oral Sci. 2020;28:91-98. doi: 10.1159/000455376. Epub 2019 Nov 7. PMID: 31940633.
5. Lombardini M, Ceci M, Colombo M, Bianchi S, Poggio C. Preventive effect of different toothpastes on enamel erosion: AFM and SEM studies. Scanning. 2014 Jul-Aug;36(4):401-10. doi: 10.1002/sca.21132. Epub 2013 Dec 11. PMID: 24374971.
6. Kanzow P, Wegehaupt FJ, Attin T, Wiegand A. Etiology and pathogenesis of dental erosion. Quintessence Int. 2016 Apr;47(4):275-8. doi: 10.3290/j.qi.a35625. PMID: 27022647.
7. Mehrabadi M, Bandani AR, Dastranj M. Salivary digestive enzymes of the wheat bug, Eurygaster integriceps (Insecta: Hemiptera: Scutelleridae). C R Biol. 2014 Jun;337(6):373-82. doi: 10.1016/j.crvi.2014.04.003. Epub 2014 May 15. PMID: 24961557.
8. Alkattan R, Lippert F, Tang Q, Eckert GJ, Ando M. The influence of hardness and chemical composition on enamel demineralization and subsequent remineralization. J Dent. 2018 Aug;75:34-40. doi: 10.1016/j.jdent.2018.05.002. Epub 2018 May 5. PMID: 29738789.
9. Nakahara T, Tominaga N, Toyomura J, Tachibana T, Ide Y, Ishikawa H. Isolation and characterization of embryonic ameloblast lineage cells derived from tooth buds of fetal miniature swine. In Vitro Cell Dev Biol Anim. 2016 Apr;52(4):445-53. doi: 10.1007/s11626-015-9987-7. Epub 2015 Dec 23. PMID: 26698579.
10. Taji S, Seow WK. A literature review of dental erosion in children. Aust Dent J. 2010 Dec;55(4):358-67; quiz 475. doi: 10.1111/j.1834-7819.2010.01255.x. PMID: 21133936.
11. Harper RA, Shelton RM, James JD, Salvati E, Besnard C, Korsunsky AM, Landini G. Acid-induced demineralisation of human enamel as a function of time and pH observed using X-ray and polarised light imaging. Acta Biomater. 2021 Jan 15;120:240-248. doi: 10.1016/j.actbio.2020.04.045. Epub 2020 May 11. PMID: 32438107.
12. Shen P, McKeever A, Walker GD, Yuan Y, Reynolds C, Fernando JR, Chen YY, MacRae CM, Schneider P, Reynolds EC. Remineralization and fluoride uptake of white spot lesions under dental varnishes. Aust Dent J. 2020 Dec;65(4):278-285. doi: 10.1111/adj.12787. Epub 2020 Aug 5. PMID: 32678914.
13. Raszewski Z, Nowakowska D, Wieckiewicz W, Nowakowska-Toporowska A. Release and Recharge of Fluoride Ions from Acrylic Resin Modified with Bioactive Glass. Polymers (Basel). 2021 Mar 27;13(7):1054.
14. Tiskaya M, Shahid S, Gillam D, Hill R. The use of bioactive glass (BAG) in dental composites: A critical review. Dent Mater. 2021 Feb;37(2):296-310.
15. Lussi A, Buzalaf MAR, Duangthip D, Anttonen V, Ganss C, João-Souza SH, Baumann T, 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 Dec;20(6):517-527. doi: 10.1007/s40368-019-00420-0. Epub 2019 Feb 14. PMID: 30762211.
16. Rošin-Grget K, Peroš K, Sutej I, Bašić K. The cariostatic mechanisms of fluoride. Acta Med Acad. 2013 Nov;42(2):179-88. doi: 10.5644/ama2006-124.85. PMID: 24308397.
17. Ganss C, Schulze K, Schlueter N. Toothpaste and erosion. Monogr Oral Sci. 2013;23:88-99. doi: 10.1159/000350475. Epub 2013 Jun 28. PMID: 23817062.
18. Ludovichetti, F.S.; Lucchi, P.; Zambon, G.; Pezzato, L.; Bertolini, R.; Zerman, N.; Stellini, E.; Mazzoleni, S. Depth of Cure, Hardness, Roughness and Filler Dimension of Bulk-Fill Flowable, Conventional Flowable and High-Strength Universal Injectable Composites: An In Vitro Study. *Nanomaterials* 2022, *12*, 1951. https://doi.org/10.3390/nano12121951

19. Ludovichetti, F.S.; Zambon, G.; Cimolai, M.; Gallo, M.; Signoriello, A.G.; Pezzato, L.; Bertolini, R.; Mazzoleni, S. Efficacy of Two Toothpaste in Preventing Tooth Erosive Lesions Associated with Gastroesophageal Reflux Disease. *Appl. Sci.* **2022**, *12*, 1023. https://doi.org/10.3390/app12031023
20. https://www.iso.org/standard/2096.html
21. Mullan F, Austin RS, Parkinson CR, Hasan A, Bartlett DW. Measurement of surface roughness changes of unpolished and polished enamel following erosion. PLoS One. 2017 Aug 3;12(8):e0182406. doi: 10.1371/journal.pone.0182406. PMID: 28771562; PMCID: PMC5542659
22. Rochel ID, Souza JG, Silva TC, Pereira AF, Rios D, Buzalaf MA, Magalhães AC. Effect of experimental xylitol and fluoride-containing dentifrices on enamel erosion with or without abrasion in vitro. J Oral Sci. 2011 Jun;53(2):163-8. doi: 10.2334/josnusd.53.163. PMID: 21712620.
23. Wiegand A, Egert S, Attin T. Toothbrushing before or after an acidic challenge to minimize tooth wear? An in situ/ex vivo study. Am J Dent. 2008 Feb;21(1):13-6. PMID: 18435369.
24. Bowen WH. The Stephan Curve revisited. Odontology. 2013 Jan;101(1):2-8. doi: 10.1007/s10266-012-0092-z. Epub 2012 Dec 6. PMID: 23224410.
25. Olivan SRG, Sfalcin RA, Fernandes KPS, Ferrari RAM, Horliana ACRT, Motta LJ, Ortega SM, Pinto MM, Deana AM, Bussadori SK. Preventive effect of remineralizing materials on dental erosion lesions by speckle technique: An in vitro analysis. Photodiagnosis Photodyn Ther. 2020 Mar;29:101655. doi: 10.1016/j.pdpdt.2020.101655. Epub 2020 Jan 7. PMID: 31923635.
26. Velo MM, Tabchoury CP, Romão DA, Cury JA. Evaluation of low fluoride toothpaste using primary enamel and a validated pH-cycling model. Int J Paediatr Dent. 2016 Nov;26(6):439-447. doi: 10.1111/ipd.12209. Epub 2015 Nov 5. PMID: 26538378.
27. Gavic L, Gorseta K, Borzabadi-Farahani A, Tadin A, Glavina D. Influence of Toothpaste pH on Its Capacity to Prevent Enamel Demineralization. Contemp Clin Dent. 2018 Oct-Dec;9(4):554-559. doi: 10.4103/ccd.ccd_667_18. PMID: 31772462; PMCID: PMC6868611.
28. Murakami C, Bönecker M, Corrêa MS, Mendes FM, Rodrigues CR. Effect of fluoride varnish and gel on dental erosion in primary and permanent teeth. Arch Oral Biol. 2009 Nov;54(11):997-1001. doi: 10.1016/j.archoralbio.2009.08.003. Epub 2009 Sep 1. PMID: 19726027.
29. Tulumbaci F, Gungormus M. In vitro remineralization of primary teeth with a mineralization-promoting peptide containing dental varnish. J Appl Oral Sci. 2020 Sep 7;28:e20200259. doi: 10.1590/1678-7757-2020-0259. PMID: 32901695; PMCID: PMC7480669.
30. Alexandria AK, Valença AMG, Cabral LM, Maia LC. Fluoride Varnishes against Dental Erosion Caused by Soft Drink Combined with Pediatric Liquid Medicine. Braz Dent J. 2017 Jul-Aug;28(4):482-488. doi: 10.1590/0103-6440201701567. PMID: 29160401.
31. da Silva BM, Rios D, Foratori-Junior GA, Magalhães AC, Buzalaf MAR, Peres SCS, Honório HM. Effect of fluoride group on dental erosion associated or not with abrasion in human enamel: A systematic review with network metanalysis. Arch Oral Biol. 2022 Dec;144:105568. doi: 10.1016/j.archoralbio.2022.105568. Epub 2022 Oct 9. PMID: 36265394.
32. Kim HN, Kim JB, Jeong SH. Remineralization effects when using different methods to apply fluoride varnish *in vitro*. J Dent Sci. 2018 Dec;13(4):360-366. doi: 10.1016/j.jds.2018.07.004. Epub 2018 Sep 1. PMID: 30895146; PMCID: PMC6388808.