Remineralizing agents: effects on acid-softened enamel

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This study sought to evaluate whether remineralizing toothpastes can protect acid-softened enamel against further erosive episodes. Fifty enamel slabs of bovine teeth with preformed erosion-like lesions were randomly assigned to 1 control and 4 experimental groups (n = 10): group 1, nanohydroxyapatite (nanoHAp) dentifrice; group 2, arginine and calcium carbonate ($CaCO_3$) dentifrice; group 3, potassium nitrate (KNO_3) and high–fluoride (F) availability dentifrice; group 4, ordinary fluoridated dentifrice (OFD); and group 5, control (deionized water). Initial hardness measurements were taken after the different treatments were applied.

Statistically significant mineral gains of 8.0% and 10.0% were exhibited in groups 1 and 4, respectively. Groups 2 and 3 showed mineral gains of 4.5% and 2.1%, respectively; these were not statistically significant. Group 5 showed mineral loss (–11.8%).

A 1-way analysis of variance showed no statistically significant differences in the mean microhardness values among groups. However, there are indications that the nanoHAp and OFD toothpastes may decrease erosive lesions after treatment, while the arginine + $CaCO_3$ and KNO_3 + F pastes may prevent the progression of erosive lesions.

Received: October 29, 2013
Revised: May 13, 2014
Accepted: May 22, 2014

As lifestyles have changed through the decades, the amount and frequency of consumption of acidic foods and drinks have increased. The wider availability and frequent consumption of acidic foods and drinks have been proposed as significant factors in the recent increase in dental erosion among patients.[1-3]

An eroded tooth surface is highly susceptible to abrasion and mechanical impacts. The interplay between erosion and abrasion (specifically oral hygiene practices) may be the main driver leading to the clinical manifestation of this disorder.[4]

The erosion process can lead to irreversible loss of ultraperipheral enamel layers. Because the remaining substrate is softened and saliva has a limited effect on this substrate, preventive measures should be initiated to reduce the erosive challenge and increase the protective oral factors, thus bringing equilibrium back to the oral environment.[5-8] From a theoretical viewpoint, elevation of calcium, phosphate, and fluoride ions in the oral fluid seems to be a reasonable strategy to promote remineralization of the eroded enamel and inhibit demineralization.[9,10]

Research has shown that the use of conventional fluoride dentifrices can reduce the dissolution of enamel exposed to the action of acids, and remineralizing agents containing calcium compounds have been introduced to the market.[11-19] The preventive action of dentifrices formulated with calcium compounds is the result of the release of calcium and phosphate into the oral environment, leading to an increased driving force for hydroxyapatite precipitation on the tooth surface.[9] Studies have shown that products containing calcium compounds can significantly increase the hardness of enamel softened by erosive substances, while reducing erosion and abrasive wear.[8,10,20-22] However, a pH cycling study found that, when acid challenges were alternated with applications of dentifrices containing casein phosphopeptide–amorphous calcium phosphate or calcium and sodium phosphosilicate, the use of these products offered no advantage over conventional fluoride toothpaste.[12]

Although studies have examined the action of toothpastes containing calcium compounds, the results were inconclusive.[23] There are no studies in the literature showing the effectiveness of toothpaste containing nanohydroxyapatite (nanoHAp) or arginine and calcium carbonate ($CaCO_3$) on dental enamel subjected to erosive challenge.[23]

Nanotechnology has been introduced to a variety of areas, including dentistry. This technology allows hydroxyapatite nanoparticles to be incorporated in various products, such as desensitizing pastes and toothpastes, which, according to the manufacturers, can be beneficial for enamel remineralization.[23,24]

Nanoparticles have higher bioactivity than conventional particles due to their small diameter and morphology, which result in an increase in surface area and improved wettability and hydration compared to conventional particles. These characteristics allow hydroxyapatite nanoparticles to release calcium and phosphate ions to the oral environment with adequate concentrations and speed to promote mineral deposition on the surface.[25,26]

Arginine and $CaCO_3$ have been incorporated in dentifrice formulations to provide the same benefit as nanoHAp, which is to promote mineral deposition above the exposed surface, leading to obliteration of dentinal tubules.[27] However, the action of arginine and $CaCO_3$ on erosion of tooth enamel has not been studied.

Numerous studies have produced a substantial knowledge database about the formation and progression of erosive lesions as well as suggested actions to delay these processes.[1,2,4,7,11,28-34] Although clinical trials continue to be the gold standard for evaluating the effectiveness of various substances on these lesions, well-controlled in vitro models can provide a valuable, fast, and effective way to evaluate the potential of new products.[5,32]

The present study was carried out to investigate the in vitro enamel remineralization efficacy of nanoHAp dentifrice, arginine and $CaCO_3$ dentifrice, potassium nitrate (KNO_3) and high-fluoride availability (F) dentifrice, and ordinary fluoridated dentifrice (OFD).

Materials and methods

Specimen preparation

Fifty permanent bovine incisors were cleaned and stored in a 0.1% thymol solution. Each tooth was sectioned at the cementoenamel junction with a low-speed water-cooled diamond saw (Isomet 1000, Buehler). Two enamel specimens (3 × 3 × 2 mm) were obtained from each coronal portion of each tooth. The specimens were embedded in acrylic resin with the enamel surface facing up and successively polished with 600-, 800-, 1200-, 1500-, 2500-, and 4000-grit aluminum oxide abrasive papers under constant water cooling. The specimens were then ultrasonically cleansed in distilled water for 20 minutes to remove polishing residue. All samples were stored at 37°C in 100% relative humidity.

Baseline hardness measurement

The specimens were measured with a microhardness tester (HMV-2, Shimadzu Corporation). With the use of a Knoop indenter, the microhardness measurements were made under a 25-g load applied for 10 seconds. Four indentations were made, with 200 µm between them, 500 µm from the right edge of each specimen. Of the 100 specimens created, 50 were selected based on their average microhardness value.

Erosion protocol

Erosion-like lesions were induced by incubating enamel slabs in 20 mL of 0.3% citric acid aqueous solution (pH 3.2) at room temperature for 2 minutes, followed by immersion in freshly made artificial saliva at 37°C for 24 hours (1 cycle). Two erosion-remineralization cycles were applied to each slab. Lesion formation was confirmed by a Knoop microhardness test. Ten enamel slabs were randomLy allocated to each of the experimental groups.

Experimental groups

The commercial toothpastes used in this study are listed in Table 1. Each experimental group was treated with a different dentifrice: group 1, nanoHAp; group 2, arginine + $CaCO_3$; group 3, KNO_3 + F; and group 4, OFD. Group 5 served as a negative control (no dentifrice; deionized water). Once every day for 6 days, a drop of artificial saliva was dispensed on each slab's surface 1 hour prior to dentifrice treatment. Dentifrices were suspended in a 1:3 deionized aqueous solution, and 20 mL of this slurry was applied to the specimen surface for 2 minutes. Following the dentifrice treatments, specimens were immersed in artificial saliva for 2 hours. The control group was unexposed to the dentifrices. Then a new erosion-remineralization (citric acid–artificial saliva) cycle was performed. The specimens were exposed to the dentifrice and erosion-remineralization cycle 6 times.

Table 1. Experimental groups, manufacturers, and basic composition of the products.

Group	Name	Product	Basic composition
1	Nanohydroxyapatite	DIO Nano-HAp Toothfoam (DIO Medical)	Nanohydroxyapatite
2	Arginine and calcium carbonate	Colgate Sensitive Pro-Relief (Colgate-Palmolive Company)	8% Arginine + calcium carbonate/ silica base
3	Potassium nitrate and high–fluoride availability	Sensodyne ProNamel (GlaxoSmithKline)	5% Potassium nitrate + high sodium fluoride availability (1450 ppm)
4	Ordinary fluoridated dentifrice	Sensodyne Cool Gel (GlaxoSmithKline)	Sodium fluoride (1200 ppm)
5	Control	Not applicable	Deionized water

Final microhardness measurement

Final indentations were made as previously described; however, measurements were taken 500 µm to the left of the measurements made at the post–lesion formation stage.

Statistical analysis

The results were analyzed using a Minitab 16 program (Minitab, Inc.). A value of $P < 0.005$ was considered statistically significant. Continuous variables were expressed as the mean and standard deviation. After homogeneity of variance and normal distribution of errors had been confirmed, a 1-way analysis of variance was performed, followed by a Tukey test at a 95% confidence level. A paired *t* test was applied to compare microhardness values at the post-erosion and posttreatment phases within each group. The percentage of microhardness loss was calculated by a formula: [(*posttreatment hardness value* – *post-erosion hardness value*) / *post-erosion hardness value*] / 100.

Results

The mean Knoop hardness number (KHN) for each group and statistical comparisons are summarized in the Chart and Table 2.

When the post-erosion and the post-treatment phases were compared, groups 1 and 4 showed greater mineral content gains (8.0% and 10.0%, respectively) than groups 2 and 3 (4.5% and 2.1%, respectively). Mineral loss was observed for the control group (–11.8%).

However, when the paired *t* test was applied to compare the post-erosion and posttreatment hardness values within each group, it was observed that only the nanoHAp (group 1) and OFD (group 4) toothpastes were able to cause a statistically significant increase in the posttreatment hardness values, whereas the arginine + $CaCO_3$ (group 2) and KNO_3 + F (group 3) products were only able to retard the development of erosive lesions.

The results of the Tukey test indicated that there was no statistically significant difference in posttreatment hardness values among the experimental groups. The only difference found was in the control group, for which no dentifrice was used.

Discussion

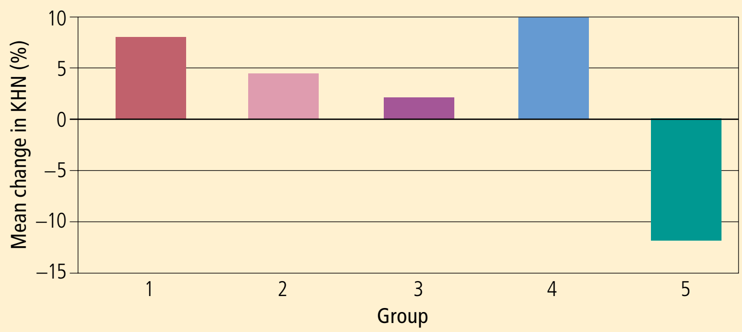
The mineral dissolution of dental enamel may not always be completely avoided, but the progression of erosive lesions can be delayed through preventive measures

Table 2. Mean (SD) Knoop surface hardness values for enamel at baseline, post-erosion, and posttreatment.

Group	Baseline	Post-erosion	Posttreatment
1	305.1 (13)	199.8 (40)[a]	215.7 (38)[aB]
2	314.8 (17)	207.3 (24)	216.5 (18)[B]
3	312.7 (13)	216.6 (27)	221.2 (24)[B]
4	314.2 (21)	191.7 (42)[a]	210.7 (20)[aB]
5	310.1 (15)	189.3 (31)[a]	167.0 (33)[aA]

[a]Statistically significant difference between post-erosion and posttreatment hardness values ($P < 0.01$). Posttreatment means with different uppercase letters are significantly different ($\alpha = 0.05$).

Chart. Change in mineralization for groups in this study.



Abbreviation: KHN, Knoop hardness number. Groups: group 1, nanohydroxyapatite dentifrice; group 2, arginine and calcium carbonate dentifrice; group 3, potassium nitrate and high–fluoride availability dentifrice; group 4, ordinary fluoridated dentifrice; group 5, control (deionized water).

taken by the patient and/or the dentist.[35-37] Thus, several products containing bioactive materials have been launched in the market as promising vehicles that release calcium, phosphate, and fluoride to the oral environment.

The microhardness test has been widely used to determine enamel surface changes.[2,4,9,11,12,20,21,38,39] With this test, the initial stages of enamel dissolution—associated with demineralization and surface weakening—can be determined.[39] Moreover, it can detect situations in which there is mineral gain on the surface, indicating remineralization of the structure.[21] Thus, positive changes in surface hardness indicate remineralization, while negative values indicate demineralization.[38]

To create the enamel erosive lesions in this study, the authors applied the same methodology described by Turssi et al, alternating immersion of the specimens in citric acid and artificial saliva.[23] The salivary film is an important factor to be considered when the progression of dental erosion is studied, as it acts as a barrier that tries to prevent the spread of acid to the tooth surface.[2] In the present study, a significant reduction in enamel hardness values was observed from baseline to post-erosion phases ($P < 0.0001$), similar to the results reported by Turssi et al.[23]

In general, positive changes in the mineral content of eroded specimens were observed after treatment with all the dentifrices used in this study. This indicates that all the toothpastes were able to prevent progression of erosion in the specimens; the negative value observed in the control group indicates progression of the erosive lesions.

Nevertheless, when a paired *t* test was applied within each experimental group (for comparison between post-erosion and posttreatment phases), results indicated that nanoHAp and OFD dentifrices not only prevented the erosive lesions from increasing but also decreased them. Possibly, the remineralization of the specimens treated with nanoHAp was due to the release of calcium and phosphate ions present in the formulation of the dentifrice, contributing to the formation of an amorphous surface layer, which may be crystallized by acquiring hydroxyl, carbonate, and fluoride from the oral environment.[40] As there are no available studies in the literature about the remineralization of erosive lesions through the use of products containing nanoHAp, the results of this study cannot be directly compared. The only studies available show that this type of bioactive material is capable of remineralizing incipient carious lesions.[9,41]

A statistically significant increase of hardness values posttreatment was found in group 4, similar to that observed in group 1. This result corroborates the data found in a recent in vitro study that showed a significant increase in hardness between specimens eroded and treated with an OFD or with a dentifrice containing calcium and phosphate.[23] Another study showed that a common OFD had a better performance on the treatment of carious lesions than casein phosphopeptide–amorphous calcium phosphate and calcium sodium phosphosilicate products.[12]

Currently, nanoHAp toothpaste is recommended for treatment of dentin sensitivity. However, as the principle of action involves mineral precipitation above the dentinal tubules, presumably a nanoHAp toothpaste could also be used as an agent for tooth enamel remineralization.[27]

There are no available studies in the literature about the use of dentifrices concurrently to erosive challenge on enamel. Future in situ and in vivo studies that take into account all oral conditions should be conducted to verify the effectiveness of these products on human enamel.

Another preventive strategy that has been widely used is the incorporation of high amounts of fluoride in dentifrices. The fluoride mechanism for retarding the progression of erosive lesions is the increased driving force for the precipitation of fluoridated hydroxyapatite (more resistant to acid challenges than hydroxyapatite).[9,42] However, in this study, the dentifrice containing high F availability (KNO_3 + F) was not able to cause a statistically significant increase in posttreatment KHN. Different results were presented in an in situ study, which observed that this same dentifrice promoted increases in post-erosion KHN.[6]

Conclusion

Within the limitations of this in vitro study, the OFD and nanoHAp dentifrices were successful with respect to remineralization of enamel erosive lesions. Arginine + $CaCO_3$ and KNO_3 + F products were only able to delay the development of erosive lesions. The nanoHAp and OFD dentifrices not only prevented erosive lesions from increasing but also caused the erosions to decrease.

Author information

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Manufacturers

Buehler, Lake Bluff, IL
800.283.4537, www.buehler.com

Colgate-Palmolive Company, New York, NY
800.226.4283, www.colgate.com

DIO Medical, Kyunggi-do, South Korea
82.31.776.3690, www.diomedical.com

GlaxoSmithKline, Research Triangle Park, NC
888.625.5249, www.gsk.com

Minitab, Inc., State College, PA
814.238.3280, www.minitab.com

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