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Remineralization Potential of a New Toothpaste Formulation: An *In-Vitro* Study

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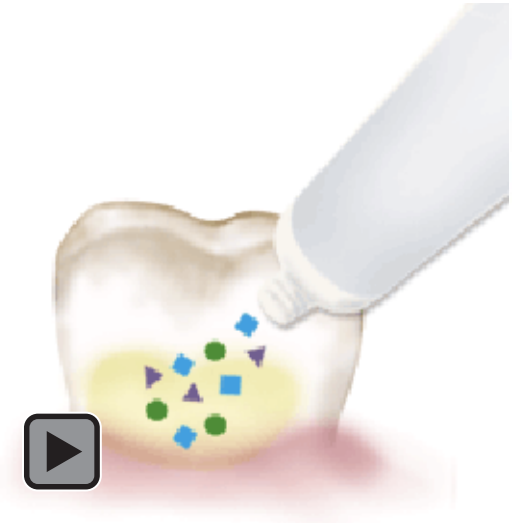

Abstract

The aim of the present study was to determine the ability of a dentifrice containing a mixture of ion-exchange resins (named NMTD), which supplies calcium, fluoride, phosphate, and zinc ions, to promote remineralization and/or inhibit demineralization of dental human enamel in a pH cycling model *in vitro*. A fluoride toothpaste was used as the control. The enamel specimens were tested for microhardness before and after 10 days and 16 days of the demineralizing and remineralizing treatments. The results of this study showed both dentifrices were effective in limiting *in vitro* enamel demineralization although the effects were not significantly different from each other. Inclusion of calcium and phosphate ion-exchange resins in the dentifrice containing a fluoride ion-exchange resin maintained a similar net outcome of the conventional dentifrice in the demineralization/remineralization process under the experimental conditions employed.

Keywords: Remineralization, toothpaste, ion-exchange, fluoride

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Introduction

Despite the fact caries affects a large percentage of the population, its rates have declined substantially partially due to fluoride use. The protective role of fluoride against dental caries was not recognized until the mid-1930s, when epidemiologic studies[1] demonstrated children drinking naturally fluoridated water had fewer caries than those in populations with water supplies low in fluoride concentration.

The presence of fluoride in saliva has been correlated with increased rates of remineralization and decreased caries incidence. Even trace concentrations of fluoride ions are effective in promoting calcium hydroxyapatite formation from supersaturated solutions of calcium and phosphate. For this reason, fluoride is added to toothpastes, mouthrinses[2], and drinking water as an anticaries agent. The use of fluoride-containing toothpastes has proven to reduce the incidence of caries in numerous clinical studies. Most toothpastes available in the United States contain about 1100 ppm fluoride.

However, fluoride's ability to promote remineralization in the oral environment is limited by the presence of calcium in saliva. Demineralization and remineralization can be considered a dynamic process, characterized by the flow of calcium and phosphate out of and back into tooth enamel, which should be balanced in order to prevent the progression of caries. This means the formation of a cavity will be prevented if the average amount of demineralization that occurs is equal to or exceeded by the average amount of remineralization. The pH at which demineralization and remineralization occurs depends on the concentration of calcium and phosphate in saliva and plaque fluid.

The new mineral that is formed if fluoride, calcium, and phosphate are present in adequate proportions, contains hydroxyapatite and fluoroapatite ($Ca_5(PO_4)_3F$), both of which are

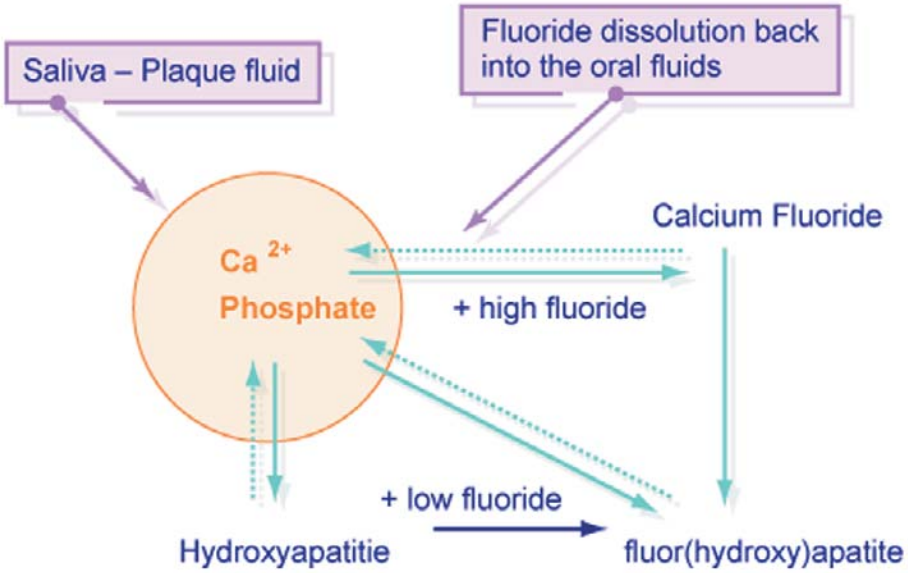
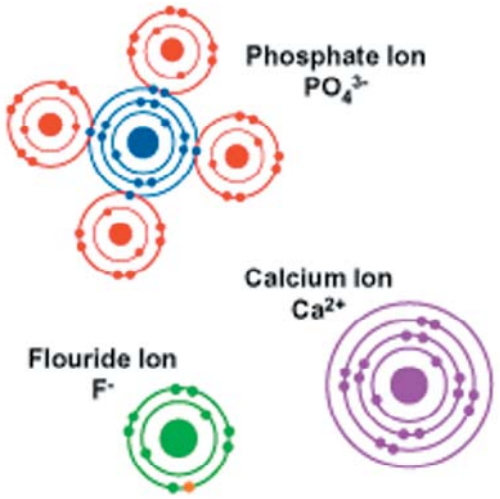


Figure 1. Some chemical reactions relevant to the caries process involving fluoride.

less soluble than the original carbonated calcium hydroxyapatite.[3] The relevant chemical reactions are schematized in Figure 1.

Probably the most cost-effective caries-preventive treatment available today is fluoridation of municipal water supplies (optimal concentration of fluoride in water ranges from 0.7-1.0 ppm) and the use of fluoride-containing toothpastes. However, with the introduction of fluoridated water in some countries and the availability of dental products containing fluoride in most countries, there has been an increase in exposure to fluoride ingestion in children and also increased risk of toxicity and dental fluorosis.[4] In this sense, the current fluoridated toothpastes have an important shortcoming that is identified by a high concentration of fluoride released into biological fluids. For this reason, rational precautions when using fluoride in children less than 6 years old will reduce any risk considerably.



Future perspectives of fluoride utilization could be found in optimizing the control and/or slow release of fluoride in the oral environment. Furthermore, the essence of the remineralizing concept might be achieved by simultaneously supplying calcium, phosphate, and fluoride ions to the teeth in order to induce the formation of calcium fluoroapatite which remineralizes and strengthens the tooth. In this sense, a toothpaste with a controlled release of mineral ions based on an ion-exchange system instead of the dispensing system that keeps the calcium source separate from phosphate and fluoride salts[5] is proposed.

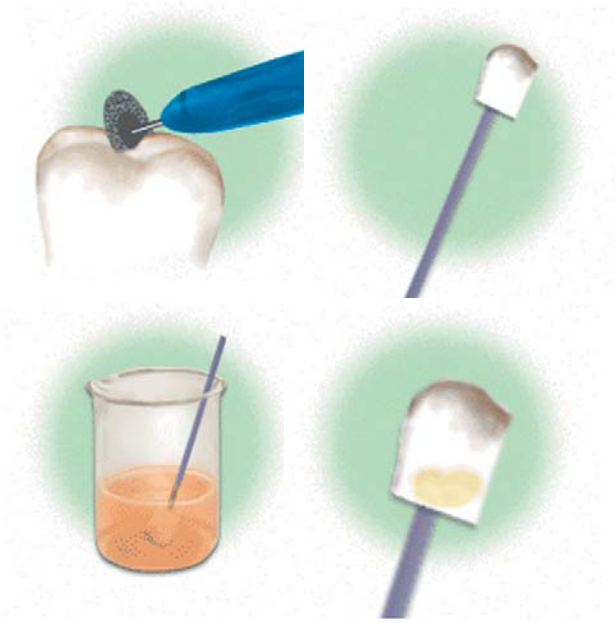
This new product allows mixing all the ions together and preventing them from chemically reacting with each other and precipitating before the application. Ion-exchange resins are insoluble high molecular weight compounds carrying ionic functional groups that can react with ions in solution through the ion-exchange mechanism.[6] The majority of these resins are not toxic and some of them are used in the food industry[7], pharmaceutical industry, and in medical applications.[8] The application of ion-exchange materials has advantages in comparison with the conventional chemical reagents. These materials do not introduce undesirable ions into the solution, ions release is carried out only by the ion-exchange mechanism, they are characterized by practically neutral pH values, and they can also adsorb bacteria on the surface. On the other hand, it provides a controlled release system for the anti-carious treatment of dental tissues. Although a long time has passed since fluoride ion-exchange resins were introduced and began being used in the dental field[9-11], no data about simultaneous release of calcium, phosphate, and fluoride by ion-exchange resins mixture in toothpaste has been found in the literature.

The aim of the present study was to determine the ability of a dentifrice containing a mixture of ion-exchange resins (named NMTD)[12] which supplies calcium, fluoride, phosphate, and zinc ions to promote remineralization and/or inhibit demineralization of dental human enamel in a pH cycling model *in vitro*.

Materials and Methods

Preparation of Carious Lesions

Enamel samples from third molar human teeth were sectioned into four parts by using a diamond disc. Each part was mounted in 0.6 cm diameter plastic rods with a dental adhesive (Optibond Solo Plus, Kerr, USA). In order to prevent adhesive from hydrolyzing the interface enamel-plastic rod, it was covered with a second dental adhesive (Optibond FL, Kerr, USA). All specimens were ground and polished, and twenty specimens presenting a perfectly flat and intact surface were selected and divided into two groups. Acid resistant nail varnish was used to cover all sides of the specimen except the surface.



Carious lesions representing the preliminary stage of subsurface enamel demineralization were produced by suspending each rod, containing each enamel specimen, into 13 ml of 0.1 M lactic acid/0.2% polyacrylic acid (Carbopol C907)/50% saturated hydroxyapatite solution at pH 5.0 for 72 hours.[13] After creating the lesion, an average surface hardness of 107±21 Knoop hardness number (KHN) was obtained (sound enamel: 310 < KHN < 350).[14]

Test Dentifrices

Two test dentifrices were compared in this study: a sodium fluoride toothpaste containing 0.16% (w/v) fluoride ion (Crest® Extra Whitening toothpaste, Procter & Gamble, OH, USA) and an experimental toothpaste based on a mixture of ion-exchange resins, named NMTD, which releases calcium, fluoride, phosphate, and zinc ions. Free ionic fluoride was evaluated from the supernatant of a centrifuged 0.4% (w/w) dentifrice:miliQ water suspension whereas total fluoride was evaluated directly from the suspension without centrifuging. Fluoride available in both toothpastes and a placebo, containing neither fluoride ion nor any ion-exchange resin, were evaluated following a modification of the hexamethyldisiloxane (HMDS) microdiffusion method developed by Taves.[15] In a polystyrene Petri dish, 1 ml of toothpaste suspension and 2 ml of miliQ water were placed. The trapping solution, 100µl NaOH 0.075N, was placed in droplet form on the lid which was next sealed with Vaseline. One ml of HMDS-saturated 3N H_2SO_4 solution was introduced into the dish through a 1 mm hole in the lid and the hole was quickly covered with Vaseline. All samples were gently hand agitated and left overnight. After this time, the trapping solution was neutralized with 50µl $HClO_4$ 0.15N and analyzed in TISAB background with a fluoride ion-selective electrode (Orion, USA).

pH-Cycling Experiments

Specimens were randomized in groups of five specimens each. The pH cycling treatment regimen was a variation of the method originally developed by Ten Cate and Duijsters.[16] It consisted of a one-minute soaking of each group in 10 ml of 33% (w/w) dentifrice/natural human saliva slurry, four times per day (at 8:30 a.m., 9:30 a.m., 2:30 p.m., and 3:30 p.m.) to simulate tooth brushing exposure. Between treatments with dentifrice, the treatment groups were immersed in 15 ml 350 rpm agitated natural human saliva (pH=7.8±0.5) at 37˚C for 1 hour periods to effect remineralization through an acquired pellicle (saliva was collected by wax stimulation each day). In order to simulate the daily acid challenges from plaque bacteria, specimens were immersed daily at 10:30 a.m. for three hours in a synthetic acid demineralization solution (same composition as lesion preparative solution). The continuous demineralization/remineralization cycles were carried out for 16 days. Over the weekend, specimens were refrigerated in a 100% humidity atmosphere.

Surface Microhardness Analysis

Three indenter penetration measurements were made initially, after demineralization, and after 10 and 16 days of treatment using a Leco M-400-H1 hardness testing machine with a 200g load. The rods containing the enamel specimens were held perpendicular to the indenter path by using a specially designed specimen holder. Knoop hardness measurements were determined from the extent of the indenter lengths.

Data Analysis

Results obtained on microhardness determination were analyzed by a one-way ANOVA test. The differences among mean values were compared by Student-Newman-Keuls multiple comparison test ($P<0.05$).


Dentifrice Soak
1 minute
Saliva Soak
1 hour
Dentifrice Soak
1 minute
Saliva Soak
1 hour
Acid Soak
3 hour
Saliva Soak
1 hour
Dentifrice Soak
1 minute
Saliva Soak
1 hour
Dentifrice Soak
1 minute
Saliva Soak
17 hours

Table 1. Fluoride availability.

Treatment group	Total fluoride (ppm)	Free ionic fluoride (ppm)
Placebo	5 ± 2	3 ± 1
NMTD toothpaste	1456 ± 126	965 ± 85
NaF toothpaste	999 ± 92	1032 ± 92

Results

Fluoride Availability

Fluoride availability results are shown in Table 1. We can see the placebo has no fluoride in its formulation whereas the new toothpaste has a considerable higher content of total fluoride than the commercial one; it is known the absence of fluoride content does not promote remineralization. The placebo formulation was not tested for the pH-cycling experiments, and only the positive control was used as reference for the comparison of results. However, it can be seen both toothpastes have a comparable level of free ionic fluoride. The comparable level of free ionic fluoride is due to the fact the particles of fluoride ion-exchange resin constituting the active principle of the new toothpaste are surrounded by a layer of soluble NaF, which was used for the fluoride ion-exchange preparation. So, when the new toothpaste enters in contact with the natural human saliva, fluoride appearance into the environment will take place through two mechanisms: first, by direct solubilization of fluoride from the NaF salt and second, through an anion-exchange process between fluoride immobilized in the organic matrix and anions present in the natural human saliva, i.e., chloride.

The results shown in Figure 2, concerning the kinetics of total fluoride available, indicate an increasing difference between the concentrations of fluoride from NMTD paste and NaF paste present in solution. This behavior corresponds to the ion-exchange mechanism present in the novel toothpaste. At the beginning of both experiments, the fluoride release is practically the same due to the same source of fluoride as indicated before and NaF was also present in the NMTD toothpaste. In the case of NMTD paste, the ion-exchange resin is encapsulated in the toothpaste base matrix, so the exchanging solution must first diffuse through the toothpaste base and then through the particles of the resin. Furthermore, it is very well known ion-exchange resins need a previous period of swelling in order to help ions diffuse through the inner paths of the ion-exchange resins[6] apart from the time of the ion-exchange reaction itself. It must be noted the mechanism that involves the fluoride releasing from the new toothpaste is complex and long-lasting.

Surface Microhardness Results

The surface microhardness evaluations of the lesions are shown in Table 2, in which the hardness numbers are indicated for initial lesions and post pH-cycling lesions. The same data is presented in Figures 3 and 4, in terms of Knoop hardness number (KHN) or percentage of remineralization versus time, respectively. Each point on each line represents the mean of the treatment of ten specimens per group. In general, fluoride dentifrices effect significant increases in surface hardness of the specimens during pH cycling remineralization conditions. See Figures 3 and 4 for verification.

Both fluoride-based toothpastes had limited lesion progression. Both types of dentifrices were not statistically different from each other ($p>0.05$), and in each case there were no significant differences after 10 and 16 days of treatment ($p>0.05$).

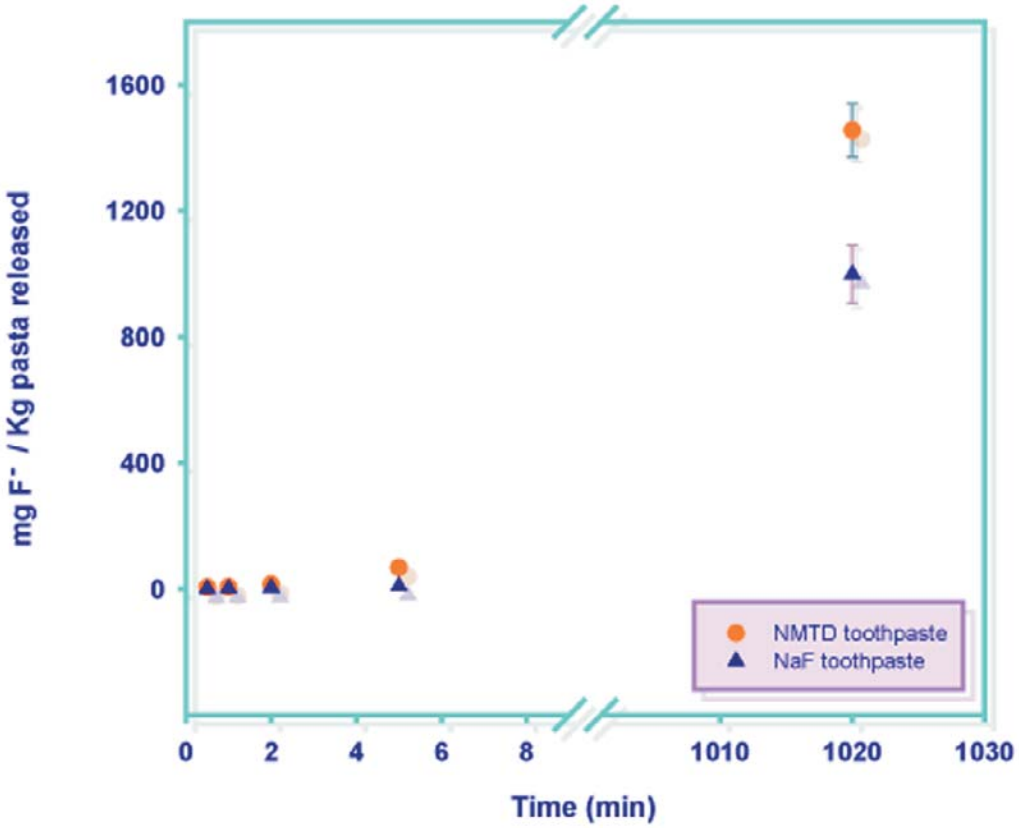


Figure 2. Kinetics of total fluoride availability.

Table 2. Enamel surface microhardness measurements.

Treatment group	KHN Initial	KHN After 10 days	KHN After 16 days
NMTD toothpaste	104 ± 9 (a)	135 ± 21 (c)	150 ± 19 (b) (c)
NaF toothpaste	110 ± 10 (a)	144 ± 19 (b) (c)	159 ± 13 (b)

(a), (b), (c) test groups not significantly different at $p < 0.05$ level

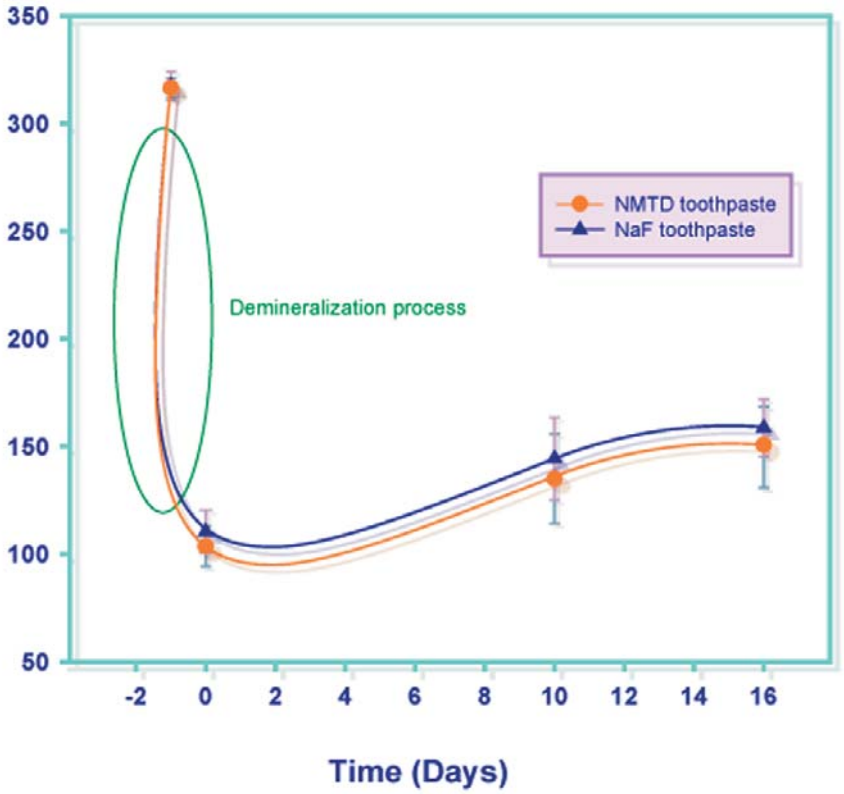


Figure 3. Enamel surface hardness evolution during the demineralization and remineralization process. Each line joins mean data points for each test group.

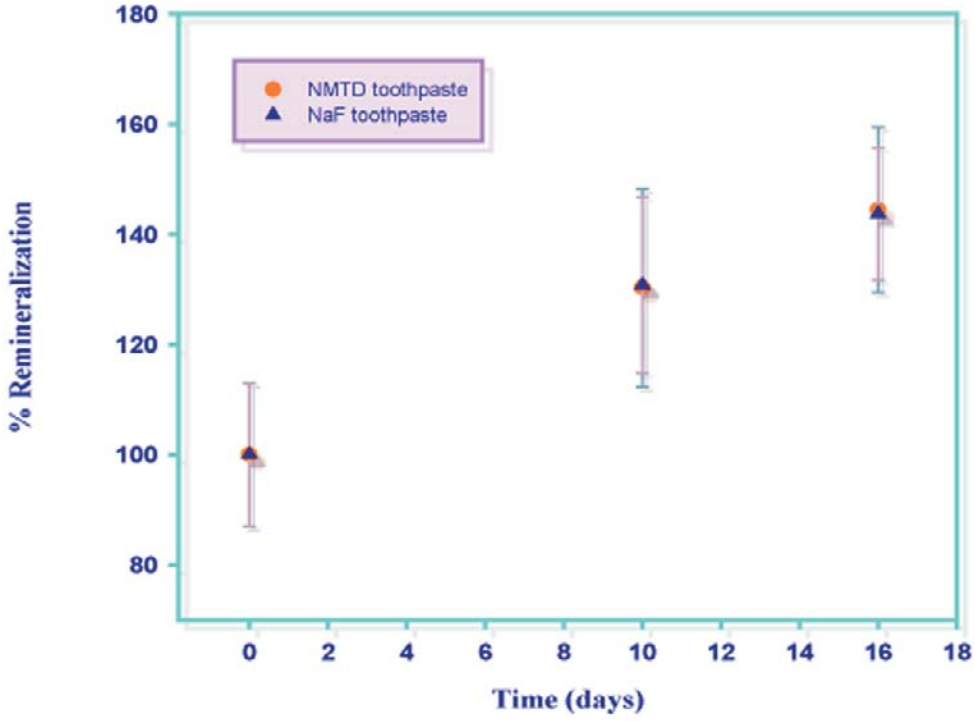
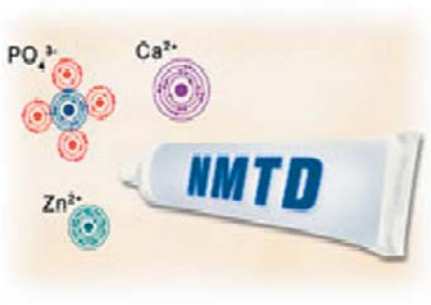


Figure 4. Remineralization percentage exerted by toothpastes tested.

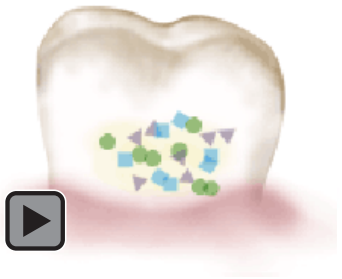
Discussion

The *in vitro* model reported here provides a test to measure the inhibition of demineralization and the enhancement of remineralization. It reveals whether the overall caries progression can be remarkably inhibited in a cycling demineralization/remineralization situation. The results of this study indicate the new toothpaste based on NMTD, having a smaller release of fluoride into saliva, achieves similar results to the commercial control NaF toothpaste. In this sense, both toothpastes inhibit demineralization and enhance remineralization as in 16 days a 50% increase in enamel hardness is achieved. These results confirm the hypotheses originated by Koulourides and coworkers indicating that when early enamel lesions are fluoride-enhanced, the fluoride provides remineralization and acid resistance to enamel.[17-19]



Free ionic fluoride supplied by the dentifrices and the fluoride's ability to incorporate into the mineral part affected by the carious lesion are mainly responsible for the remineralization potential of both toothpastes. Note the new toothpaste also supplies calcium, zinc, and phosphate ions. The role of the zinc ion is anti-bacteriological, that is, prevention from the formation of plaque acid. On the other hand, treatment of enamel with calcium and phosphate has been shown to promote fluoride uptake by enamel.[20-21] A higher fluoride content will reduce enamel solubility due to hydroxyapatite transformation to fluoroapatite by OH-/F- substitution, and the dissolution of calcium hydroxyapatite in an acidic media is a reversible reaction, the direction of which is dependent on the pH of the solution and the concentrations of calcium and phosphate ions present. This way, during remineralization the inclusion of higher concentrations of ionic calcium and phosphate in the media will result in an increase in the degree of supersaturation and, thereby, an increase in the rate of deposition of mineral into the enamel.

The novelty of the product assayed, is that it is capable of supplying calcium, phosphate, and fluoride ions through a controlled release mechanism depending on the tooth demand. In this sense, if a tooth has a mineral defect due to an acidic attack, undergoing a carious lesion, the product is able to regenerate the natural composition of the tooth as well as to regulate the pH conditions in order to prevent calcium hydroxyapatite dissolution.



Concerning the content of calcium and phosphate ions in the new toothpaste, it is lower than the ones present in the experimental toothpaste assayed by Schemehorn et al., which concluded supplementation of salivary calcium and phosphate concentrations with a fluoride-containing dentifrice promotes the remineralization of enamel.[2] It must also be considered the *in vitro* cyclic regimen of treatment may underestimate the protective effects of the toothpaste because the enamel specimens are thoroughly rinsed after each treatment and any residual toothpaste is washed away. Under these conditions the concentration of fluoride, calcium, and phosphate ions in the media was not optimized, especially since the specimens were brushed for only one minute as it is done clinically. The presence of NaF in the corresponding resins mixture of NMTD met some of those conditions, as observed by the results. Future work with the absence of NaF in the resin, will contribute to clarify the mechanism of fluoride release in such complex systems. Taking into account these considerations and the parameters influencing the ions released from a resin (i.e., strong/weak character, particle size, available surface, etc.), further studies should be carried out to complete a methodical knowledge of this system. At this point, we suggest a revision of calcium and phosphate ion content in the dentifrice and further demineralization/remineralization in both *in vitro* and *in vivo* studies.

Conclusions

The new toothpaste containing NMTD which supplies calcium, fluoride, phosphate, and zinc ions was effective in limiting *in vitro* enamel demineralization. Inclusion of calcium and phosphate ion-exchange resins in the dentifrice containing also a fluoride ion-exchange resin, did not affect the net outcome of the demineralization/remineralization process under the experimental conditions employed.

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