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Comparison of the remineralizing effect of a sodium fluoride mouthrinse versus a sodium monofluorophosphate and calcium mouthrinse: An in vitro study

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Abstract

Objectives

The aim of this in vitro study was to compare the remineralizing effect of three rinses: (A) 0.17% sodium monofluorophosphate and 0.05% calcium glycerophosphate (220 ppm fluoride), (B) 0.05% sodium fluoride (220 ppm fluoride) and (C) control mouthrinse (without fluoride or calcium).

Method and Materials

DeminerIALIZED areas were created in 90 pieces of bovine enamel by submerging them in an acid solution (pH 4.4) for 48 hours. Part of the surface was painted with nail varnish to preserve the demineralized area and the specimens were assigned at random to three groups. The specimens were stored in artificial saliva at pH 7 and 37°C for 30 days. Twice daily they were immersed for 60 seconds in the respective mouthrinse. Following the experimental period, the specimens were bisected and examined by scanning electron microscopy.

Results

The mean percentage of remineralization produced by the mouthrinses was as follows: (A) 54.08 (95% CI 46.37-61.78), (B) 38.43 (95% CI 30.89-45.98) and (C) 30.18 (95% CI 25.56-34.80). The differences between the three groups were statistically significant.

Conclusion

The results show that the fluoride and calcium mouthrinse has a significantly greater remineralizing capacity than the fluoride mouthrinse with the same fluoride ion concentration under the in vitro conditions of this study.

Key words: *Demineralization, remineralization, fluoride, mouthrinse, bovine enamel, calcium glycerophosphate.*

Introduction

In the oral cavity, changes in the mineral content of the teeth regularly occur. Under normal conditions, the losses and gains balance out. However, if the balance shifts towards demineralization, which can occur for a number of reasons, and if this situation continues over a period of time, a caries lesion forms. It is very important to detect and treat caries in the early stages to avoid the continuing loss of minerals and prevent the lesion from becoming cavitated. Early caries diagnosis allows the lesion to be treated medically by applying remineralizing agents (1).

In 1942, Dean demonstrated the inverse relationship between the fluoride concentration in the drinking water and caries experience. Since then, this important discovery has been confirmed by a number of epidemiological studies conducted in other countries (2).

The first theories concerning the mechanism of action of fluoride were based exclusively on its pre-eruptive effect. However, later clinical examinations showed that children whose teeth were already formed and mineralized experienced a significant reduction in dental caries on moving to a fluoridated area. Arnold, in 1957, is probably the first author to have mentioned the post-eruptive effect of fluoride in the drinking water and, consequently, the ability of topical fluoride to reduce the incidence of caries (3).

The anti-caries effectiveness of a frequent use of preparations containing low concentrations of fluoride, such as toothpastes and mouthwashes, has been demonstrated. The World Health Organisation recommends the use of mouthrinses as an alternative caries prevention and treatment method (4).

Recent years have seen the appearance of preparations that combine fluoride with other chemical agents to improve its efficiency, increasing its capacity to remineralize incipient caries. One such compound is calcium glycerophosphate (CaGP). Studies have established the anticaries properties of CaGP and it has been added to toothpastes, generally in combination with sodium monofluorophosphate (5). However, no studies have been made to assess the efficacy of a mouthrinse comprising CaGP and sodium monofluorophosphate.

The aim of this in vitro study was to compare the remineralizing effect on incipient caries lesions of a mouthrinse containing 0.17% sodium monofluorophosphate and 0.05% calcium glycerophosphate (220 ppm fluoride), a 0.05% sodium fluoride mouthrinse (220 ppm fluoride) and a mouthrinse without fluoride or calcium employed as a control.

Material and Methods

- Sample preparation

90 pieces of enamel measuring approximately 5 x 4 x 3 mm were obtained from 30 bovine incisors. The sample

inclusion criteria were as follows: permanent incisors having fully formed roots and minimum incisal wear. The pieces were hand-cut at a speed of 15,000 rpm, without refrigeration, using Komet® 918PB-104-220 diamond discs mounted in a hand piece. The resulting pieces were embedded in acrylic resin (Forestadent® powder/liquid refs. 401-0010 and 402-0010). The surface of the enamel was then polished with water and polishing discs of different grain sizes: 220, 320 and 400 (3M Wetordry®).

- Demineralization

A third of the surface of each specimen was coated with blue acid-resistant nail varnish so that part of the original enamel would remain intact. Demineralization lesions were created in the remaining two thirds by submerging the enamel specimens in 1 litre of an acid solution composed of 2.2 mM Ca²⁺ (as Ca(NO₃)₂), 2.2 mM PO₃⁴⁻ (as K₂HPO₄) and 50 mM of acetic acid (6) for 48 hours (pH 4.4; 37°C). The pH and temperature were recorded using the Hanna pH 211® benchtop pH meter. At the end of the demineralization period, the specimens were washed well with distilled water and the middle third of the surface was painted with red nail varnish to preserve the initial demineralization lesion, leaving the last third uncovered for exposure to the rinse during the remineralization stage. The 90 specimens of enamel were assigned at random to three groups of 30 samples each (A, B and C).

- Remineralization

During this stage, the samples of enamel were immersed in a bath of artificial saliva composed of 0.7 mmol/l CaCl₂, 0.2 mmol/l MgCl₂, 4 mmol/l KH₂PO₄, 30 mmol/l KCl and 20 mmol/l Hepes (7), renewed every 48 hours. They were kept at 37°C and pH 7 for 30 days, with constant circulation (8). Each group was exposed to a different mouthrinse, with the following compositions:

- Group A: 0.17% sodium monofluorophosphate mouthrinse (220 ppm fluoride) and 0.05% calcium glycerophosphate

- Group B: 0.05% sodium fluoride mouthrinse (220 ppm fluoride)

- Group C: control mouthrinse (0 ppm fluoride)

Each group was immersed in 200 mL of the respective mouthrinse twice a day for 60 seconds, then well rinsed with distilled water and returned to the artificial saliva bath. The mouthrinse was renewed daily. The study design was single-blind: the researcher who conducted the experimental work was unaware of the composition of the mouthrinses throughout the procedure.

- Measurements

Following the remineralization stage, each of the specimens was perpendicular bisected to obtain two sections, each showing the three thirds: the anterior third with healthy enamel, the middle third with the initial demineralization lesion and the posterior third with the residual demineralization lesion following exposure to

the mouthrinse during the remineralization stage. Each of the 180 sections obtained from the 90 specimens of enamel was homogeneously polished with abrasive paper discs (3M Wetordry® 220, 320 and 400) and labelled. The sections were sputter-coated with gold and palladium (Sputter Coater BIORAD® SC-500) and observed by scanning electron microscopy (HITACHI® S-2500) at 10 kV. A representative image of the initial demineralization lesion (demineralization stage) of each section and another of its residual demineralization lesion (remineralization stage) were obtained at x500 magnification. Five measurements were taken on the same points for each image with the QUARZ digital image capture system. Consequently, each section provided five measurements of initial demineralization and five of residual demineralization in µm.

- Data analysis

Only those sections where both initial demineralization and residual demineralization could be clearly quantified were considered valid (153 sections). The mean initial demineralisation and mean residual demineralisation for each section were obtained from the measurements of the images.

In order to determine whether the remineralization (difference between initial and residual demineralization) of the samples was significant in each of the three groups, a Student's t-test was used to compare the mean initial demineralization and mean residual demineralization of paired groups.

The remineralization percentage was also calculated as the quotient of remineralization divided by initial demineralization, multiplied by 100.

In order to determine which of the three groups showed the greatest remineralizing efficiency, a one-way ANOVA test was used to compare the mean remineralization percentages. All statistical tests were carried out for a 95% significance level and p<0.05.

The data were analysed with SPSS v13.0® statistical software (SSPS Inc., Chicago, USA).

Results

Table 1 shows the size of the valid sample, mean initial demineralization, mean residual demineralization and mean remineralization for each group. The differences between the three groups are statistically significant. Fluoride and calcium rinse has shown a remineralisation with a 95% confidence interval between 46.37-61.78%, versus fluoride rinse (30.89-45.98%), and control rinse (25.56-34.80%). The percentages of remineralisation of the initial lesion of the three rinses are shown in figure 1. The differences in mean remineralization (in %) between the three groups determined by a one-way ANOVA test are statistically significant. On contrasting the three groups post hoc, it will be seen that the mean remineralization percentage for group A is highly significant in comparison to both group B and group C and that the difference between groups B and C is also significant (table 2).

Table 1. Mean initial demineralization, residual demineralization and remineralisation by group.

Group	Initial demineralization (Demineralization stage)	Residual demineralization (Remineralization stage)	Mean Difference (Remineralization)
Group A: F+Ca rinse (n=39)	47.86 [43.87-51.86]	22.91 [18.29-27.54]	24.95* [21.48-28.41]
Group B: F rinse (n=27)	51.40 [47.40-56.50]	33.22 [27.18-39.27]	18.72* [15.42-22.03]
Group C: Control rinse (n=37)	59.52 [50.26-68.78]	43.07 [34.76-51.38]	16.44* [14.17-18.71]

Values for depth in µm.
*Paired difference t test; p < 0.05
[] 95% Confidence Interval

Table 2. Inter-group comparison of mean percentages of remineralisation.

Post-hoc contrasts between groups	Difference in means	t statistic	Significance
Group A (F+Ca rinse) versus Group B (F rinse)	54.08 versus 38.43	2.84	0.006*
Group A (F+Ca rinse) versus Group C (Control rinse)	54.08 versus 30.18	5.39	0.00*
Group B (F rinse) versus Group C (Control rinse)	38.43 versus 30.18	2.007	0.049*

One-way ANOVA test, * =p<0.05

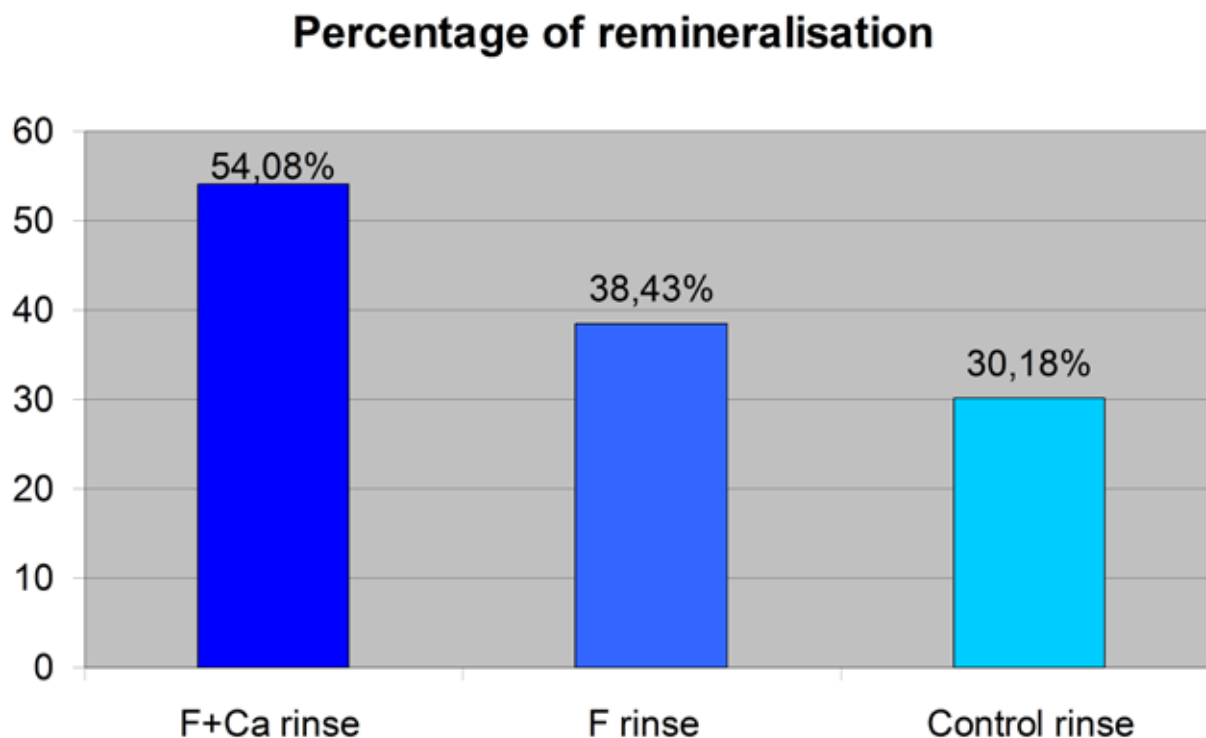


Fig. 1. Percentage remineralisation by group.

Discussion

Numerous in vitro studies have assessed the remineralizing capacity of fluoride and other remineralizing agents, including calcium glycerophosphate, employing both human and bovine teeth. Edmunds et al. (9) demonstrated the resemblance between bovine and human enamel. The use of bovine enamel presents a number of advantages compared to human enamel, as while the microscopic structure is very similar, the distance from the surface to the amelodentinal junction is greater, allowing the surface to be polished to remove irregularities with no risk of the remaining enamel being insufficient. Additionally, nowadays, ethical restrictions and greater access to dental care have made it more difficult to obtain human teeth in good condition for study purposes (9). Many authors have employed bovine enamel for studies of adhesion (10), demineralization (6,11) and remineralization (12-14).

Different demineralizing solution compositions are found in the literature. Most are composed of calcium and phosphate together with acetic acid or lactate. The main differences lie in the concentration of each component, which influences the final pH, and the sample exposure time. The pH employed ranges from 3.2 to 5 and the time varies between two hours and 21 days. The higher pH values are generally used with longer exposure times, but both parameters depend on the desired

result. In this study, demineralization similar to an initial caries lesion was sought, in other words, loss of mineral content without loss of substance, maintaining the surface of the enamel intact. An intermediate pH solution (4.4) was therefore employed for 48 hours. The composition was identical to that used with different exposure times in a study of fluoride and demineralization by ten Cate and Duijsters (6) and later by Donly et al.(15) when studying the effect of fluoride-releasing materials on interproximal caries. Chow et al.(16) used a very similar solution, although slightly more acid (pH 4.3), and an identical exposure time (two days) in their study of remineralization with a fluoride mouthrinse. As the depth of the lesions created by that protocol was between 100 and 150 μm , it was considered advisable to use a slightly higher pH (4.4).

Synthetic saliva has been used in the great majority of the studies, although authors such as González-Cabezas et al. (1) have used human saliva. The use of stimulated human saliva for this study was mainly ruled out on the grounds of its instability. Leung and Darvell (17) state that for this reason it is inappropriate for use in standardized in vitro studies. Further reasons included its being difficult to obtain and store. In the case of artificial saliva, a very large number of formulas have been employed for in vitro studies. Certain components are found in the majority of these formulas, such as the K^+ ,

Na⁺, Ca²⁺, Cl⁻ and PO⁴⁻ ions, and their pHs range from 6.7 to 7.2. The composition employed in this study was the same as that used by Eisenburger et al. (7) in their study of the effect of time on the remineralization of enamel by saliva, which had a pH of 7. The decision to renew the artificial saliva every 48 hours was based on the procedure employed by Marinelli et al. (8).

All of the many methods to quantify demineralization and remineralization present advantages and drawbacks. Using microhardness to analyse the results was ruled out for this study as this method has been little used by the authors and is somewhat obsolete for this purpose. Terahertz pulse imaging (TPI) is a method that has appeared recently and is presented as a non-invasive and non-ionising technique. However, studies of this method are still attempting to relate the refraction index with the mineral content (18). Polarised light microscopy and microradiography require a considerable loss of mineral substance before detection is possible (11). Confocal laser microscopy presents a number of advantages over other techniques. Furthermore, studies have been conducted to correlate the results obtained with other more established methods (1,19). However, few studies have quantified remineralization using this technique. Consequently, the sample examination method chosen for this study was scanning electron microscopy. This technique has been employed by a number of authors to assess both demineralization and remineralization (4,7,13). Harding et al. (13) studied samples in a scanning electron microscope without having coated them so that they could be observed again if necessary once the study had ended. In most studies, however, the samples are covered with metals such as gold or palladium to improve the quality of the images. Consequently, in this study the samples were coated prior to observation in the scanning electron microscope.

Numerous studies have already shown that daily use of a mouthwash containing a low concentration of a fluoride such as 0.05% sodium fluoride has beneficial effects, both through its preventive effect and through its remineralizing action on incipient lesions. Nonetheless, combinations of fluoride with other compounds are currently being tested with the aim of achieving better results.

The mouthwash containing fluoride and calcium presents a greater remineralizing capacity than that achieved by the fluoride mouthrinse. Although the two mouthrinses contain two different fluoride compounds, both have the same fluoride ion concentration (220 ppm). Because of this, the differences in the results can be attributed to the addition of calcium glycerophosphate. The results are similar to those obtained by Chow et al. (20) in an *in situ* study comparing a mouthrinse composed of calcium and 228 ppm of fluoride with a further two containing 250 and 1000 ppm of fluoride respectively. The fluoride

and calcium rinse achieved higher remineralization figures than those obtained with the NaF mouthrinse containing 250 ppm of F⁻, while no significant differences were found in respect of the NaF mouthrinse with 1000 ppm F⁻. An *in vitro* study by Takagi et al. (21) compared an experimental low-fluoride, calcium and ethanol rinse with other rinses containing different concentrations of fluoride and with a placebo. The results showed that the remineralizing capacity of the experimental mouthrinse was similar to that of the high fluoride concentration rinse. Chow et al. (16) subsequently studied the same fluoride and calcium rinse, comparing it with another composed of fluoride and with a placebo. The two-component rinse was the one to show the greatest efficiency. One of the advantages of this discovery is that better remineralisation results can be obtained without needing to increase the fluoride concentration, thus minimising the risks from accidental ingestion by children (16).

The remineralizing effect of the control mouthrinse (0 ppm F⁻) has been observed in other studies (16). In an *in vitro* study, Eisenburger et al. (7) concluded that exposure to artificial saliva repairs the damaged enamel: although the original structure of the healthy enamel is not restored, a mineral deposit is observed in the demineralized zone. For this reason, remineralization by the control mouthrinse could be attributed to the samples having been submerged in artificial saliva over the 30 days of the experimental period.

Conclusions

In conclusion, it may be stated that the fluoride calcium mouthrinse showed a significantly higher remineralizing capacity than that observed for the fluoride mouthrinse with the same fluoride ion concentration. As it is difficult to extrapolate the conclusions reached in this *in vitro* study to the situation in the oral cavity, *in vivo* studies need to be made in order to verify these results.

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