Revista Brasileira de Odontologia
Brazilian Journal of Dentistry
Associação Brasileira de Odontologia - Seção Rio de Janeiro
DOI: http://dx.doi.org/10.18363/rbo.v77.2020.e1783

Original Article/Cariology

# Effect of High-Fluoride Dentifrice on the Enamel Demineralization Related in Concern to Microhardness

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• **Conflicts of interest: none declared.**

## Abstract

**Objective:** to evaluate the effectiveness of high-fluoride dentifrice in decreasing the process of enamel demineralization. **Material and Methods:** thirty samples of bovine enamel were confectioned, sanded, polished and tested for determining their initial Knoop hardness. The samples were randomly divided into 3 groups: PD – Placebo Dentifrice, CD – Conventional Dentifrice (1450 ppmF) and HFD – High-Fluoride Dentifrice (5000 ppmF). To evaluate the dynamic process of demineralization, each group was subjected to pH cycling with demineralizing and remineralizing solutions. The samples were immersed for 6 and 18 hours respectively in those solutions for 5 days at 98.6ºF. The dentifrice corresponding to each group was diluted (1:3) and the samples were treated for 1 minute, twice a day. After cycling, a final microhardness analysis was performed, using ANOVA and Tukey's test ($p$<0.05). **Results:** the results showed that the hardness of the final HFD (247.4±39.4) compared to CD (211.9±53.5) showed no statistical difference ($p$≤0.05). **Conclusion:** according to the results, it can be suggested that the high concentration of fluoride in dentifrice exhibited superior results in inhibition of enamel demineralization process than conventional dentifrice.

**Keywords:** Demineralization, Enamel, Toothpaste.

## Introduction

The use of fluoridated toothpastes has greatly contributed to the decline of dental caries in industrialized countries.[1] In addition, it is considered the most rational way to use fluoride because it combines periodic interruption of biofilm by mechanical removal with the therapeutic interference of fluoride in the caries process. The mechanical removal of dental plaque, together with the regular use of toothpastes that ensure high fluoride concentrations at the biofilm-enamel interface, has resulted in a decline in caries prevalence.[2]

Typically, the fluoride concentration in toothpastes is between 1000 and 1500 ppmF. However, high-fluoride dentifrices, such as 5000 ppmF dentifrices, have been shown to be more effective in decreasing the prevalence of caries, remineralization of enamel-dentin, and interfering with the demineralization process when compared to regular dentifrices.

Baysan *et al.*,[3] for example, compared the ability to reverse primary caries lesions of two dentifrices containing sodium fluoride, one with 5000 ppm fluoride and the other with 1100 ppm fluoride. The results revealed that the group that used the 5000 ppm fluoride toothpaste after three and six months showed better results than the other group in terms of hardness, cavitation, gingival margin distance, lesion area and dental plaque accumulation.

Nordström and Birkhed,[4] likewise, compared a toothpaste containing 5000 ppm fluoride to a toothpaste with 1450 ppm fluoride in adolescents with active caries. They concluded that the 5000 ppm fluoride toothpaste had a greater impact on individuals who did not use toothpaste regularly or brush their teeth twice a day.

Thus, despite the existence of studies with positive results, there is still no consensus among countries in the indication of toothpastes with 5000 ppm fluoride regarding the control of the disease, even in groups with a high index of dental caries. In addition, there is concern about increased fluorosis after regular use of 5000 ppmF for long periods. Understanding that the literature still needs further studies regarding these dentifrices, the objective of this study was to evaluate the effect of high-fluoride dentifrice on the inhibition of dental enamel demineralization in relation to its microhardness.

## Material and Methods

### Experimental Design

Thirty specimens were made from bovine teeth (Frigorífico Mondelle, Bauru, São Paulo, Brazil), which were analyzed for initial Knoop microhardness. The specimens were taken to pH cycling, as recommended by Queiroz,[5] according to stipulated treatments. The results were obtained through the analysis of the final Knoop microhardness values and the specimens were also analyzed by surface roughness.

### Design of Enamel Blocks

For the preparation of the specimens, an Isomet electric saw (Buehler, Lake Bluff, Illinois, USA) equipped with a previously adjusted double-sided diamond blade was used. Two diamond discs were placed on the cutter, separated by a 4.0 mm spacer, then a longitudinal cut was first made on the crown and then the coronary remnant was repositioned, and a new longitudinal cut was made, resulting in a 4x4x3 mm bovine enamel block at the end.

To avoid cracking of the enamel, the cuts were made under refrigeration, thus the specimens were always moistened to avoid cracking.

**Sanding and Polishing Steps**

For sanding, a model 40-7618 polishing machine (Buehler, Lake Bluff, Illinois, USA) was placed with a 400-grit metallographic sandpaper (ER 15004, Erios, Sao Paulo, Brazil) on the turntable. The faucet was turned on in order to guarantee a steady stream of water in the sandpaper. The machine was started at low speed using two weights for 30 seconds. Firstly, the dentin block was positioned with the work face facing down so that the height was obtained, each block was approximately 3.0 mm high. Then the dentin block was repositioned with the work face up (reassembled with sticky wax) without placing wax on the surface between the block and the acrylic plate to achieve maximum parallelism between them.

The enamel was worn with 400 grit sandpaper (Erios) with one weight for 15 seconds at low speed. Then, the 600-grit sandpaper (ER 15006, Erios, São Paulo, Brazil) was used for 15 seconds, also with low speed weight and after that, in the same way and time, the 1200 sandpaper was used. (ER 15012, Erios, Sao Paulo, Brazil). The sanding was performed under refrigeration. After the sanding step, the blocks were polished with a polishing felt paper disk (Extec Corp 17606-PSA, Extec Multi-Cloth, Enfield, USA) and diamond paste (ER 20003 - 1 micrometer, Erios, São Paulo, Brazil) at low speed, using two weights for 30 seconds each. At this stage, no cooling was necessary, but only a sufficient amount of diamond suspension to fully dampen the felt paper. After polishing with the diamond suspension, the specimens were washed in hot water. Afterwards, the enamel blocks were washed in running distilled water and kept in a humid environment (Figure 1).

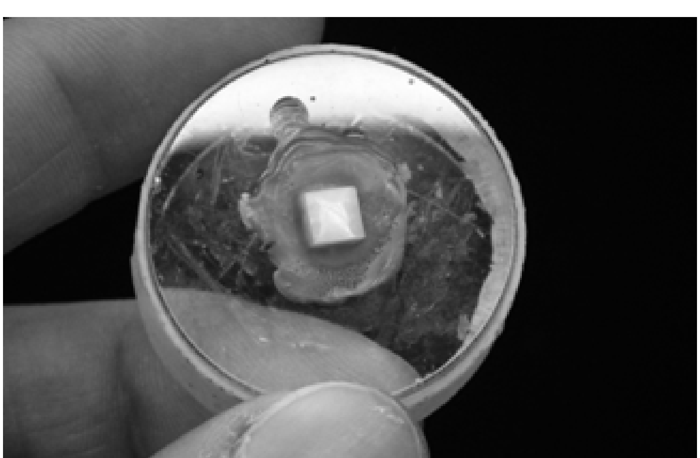

**Figure 1**. Enamel blocks sanded, polished and clean.

**Enamel blocks sanded, polished and clean**

**Initial Microhardness Analysis**

For the initial microhardness analysis (Baseline), a microhardness meter (300, Future-Tech, Tokyo, Japan) was used, with a Knoop-type penetrator and a static load of 49 grams for 5 seconds. Five separate indentations were performed for a distance of 100 µm. The indentations were made on the enamel surface in each dental block.

**Experimental groups**

After the initial microhardness, the dental samples were randomly distributed in three experimental groups, according to the dentifrices:

Group PD - Placebo Dentifrice (Malvatrikds® without fluoride, Daudt Laboratory, Rio de Janeiro, Brazil)

Group CD - Conventional Dentifrice (Colgate Original® 1450 ppmF, Colgate-Palmolive Company, Sao Paulo, Brazil)

Group HFD – High-Fluoride Dentifrice (Colgate PrevDent® 5000 ppmF, Colgate-Palmolive Company, Sao Paulo, Brazil)

**pH Cycling**

The dental samples underwent to pH cycling for five days. They were immersed in a demineralizing solution (1.28 mM Ca, 0.74 mM P, 0.05 M acetate buffer, pH 5.0) for 6 hours. After this time, the samples were washed with distilled water and immersed in a suspension of toothpaste diluted in distilled water (proportion of 1:3) according to each experimental group for 1 minute, after which the blocks were washed again with distilled water and thus immersed in remineralizing solution (1.5 mM Ca, 0.9 mM P, 0.15 M KCL, 0.1 M Tris buffer, pH 7.0) for 18 hours at 37°C. At the end of remineralization, the toothpaste treatment process was repeated, being completed twice a day.

**Final Microhardness**

After performing the treatments according to the respective groups, the microhardness in the specimens was again evaluated in order to compare with the initial microhardness. Final microhardness was evaluated in the same way as initial microhardness.

**Statistical Analysis**

After obtaining the surface microhardness values, the averages of each group were calculated. Analysis of variance (ANOVA) and Tukey test were applied to compare surface microhardness and the percentage of surface hardness loss between the different groups and at a significance level of 5%. The statistical program used was StatPlus.

## Results

The results showed that after the confection and random distribution of enamel blocks in the three different groups, the mean values of the initial surface microhardness did not show significant differences ($p \geq 0.05$) (Figure 2).

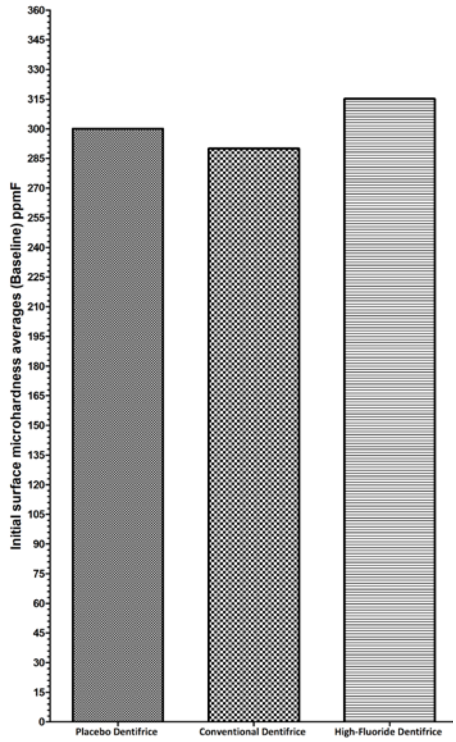


**Figure 2**. - Initial surface microhardness averages (Baseline)

**Initial surface microhardness averages (Baseline)**

After pH cycling and dentifrice application, the results showed that the surface microhardness of the CD and PD groups were not statistically different from each other ($p \geq 0.05$), but when they were compared to the HFD, a significant difference was observed ($p \leq 0.05$) (Figure 3).

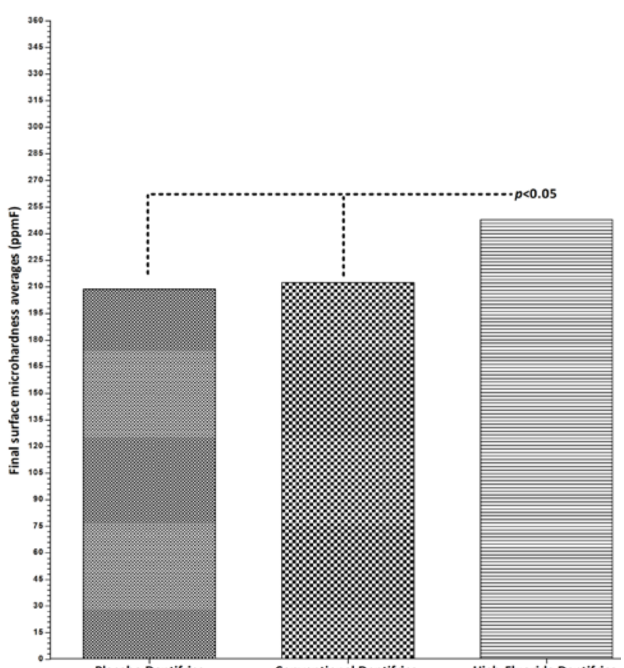


**Figure 3**. Final surface microhardness averages. Significant difference ($p \leq 0.05$) was observed when PD and CD were compared to HFD. PD – Placebo Dentifrice, CD – Conventional Dentifrice and HFD – High-Fluoride Dentifrice.

**Final surface microhardness averages**

The data obtained in each sample by Knoop microhardness analysis, before and after treatments with different toothpastes, allow the percentage of Surface Hardness Loss (SHL% / %SHL) to be calculated using the following formula shown in Figure 4.

$$\textbf{S}\text{urface }\textbf{H}\text{ardness Loss }(\textbf{SHL}) = \frac{\text{Final Hardness} - \text{Initial Hardness} * 100}{\text{Initial Hardness}} = \%$$

**Figure 4.** Percentage formula for Surface Hardness Loss (SHL%/%SHL).

The results indicate that the SHL percentage among the three experimental groups (PD, CD and HFD) was statistically different from each other ($p \leq 0.05$). However, a significant difference ($p \leq 0.05$) was observed when PD and CD were compared to HFD (Table 1).

**Table 1**. Percentual Surface Hardness Loss (SHL% / %SHL).

| Groups | SHL% / %SHL |
|---|---|
| PD | - 50.2$^{a}$ |
| CD | - 41.0$^{b}$ |
| HFD | - 27.5$^{c}$ |

ANOVA and Tukey test: Different letters indicate significant differences between groups ($p$=5%).

## Discussion

The results of the present study revealed the effectiveness of the fluoride ion in interfering with the enamel demineralization process. The study involving the three fluoridated toothpastes of different concentrations (5000 ppm ─ test group, 1500 ppm ─ control group and 0 ppm ─ negative control group) showed different efficiencies compared to the negative control group, as well as the difference between toothpastes above 1500 ppm when the surface hardness loss is evaluated.

The possibility of interfering with enamel dissolution, thus significantly reducing mineral loss and reversing initial carious lesions, makes the fluoride ion an important element for the preservation of dental health, in view of this anti-cariogenic property.[1]

The model designed in the present work, which is in accordance with the methodology applied by Queiróz,[5] was able to simulate the enamel demineralization process by pH cycling and also reproduced the toothpaste treatment similar to that commonly used in the mouth during brushing.

Especially regarding the action of dentifrices evaluated in this study, *in vitro*, it was found less enamel demineralization in the presence of fluoride when compared to the absence of the substance. The results of the enamel surface hardness analysis proved the effect of the treatments, reducing the demineralization of the initially healthy enamel blocks. Bizhang[6] found that all fluoridated dentifrices had significantly better results in reducing demineralization when compared to the placebo group (without fluoride). Results observed by Mathaler[7] revealed lower surface hardness loss in enamel treated with fluoride dentifrices after demineralizing challenges compared to the placebo group.

Another subject of discussion among the authors is the effect of high-fluoride dentifrices on the reversal of root caries. In this context, Srinivasan *et al.*[8] assure that the application of a 5000 ppm fluoride dentifrice to adults twice daily significantly improves the surface hardness of root caries lesions when compared to the use of a 1350 ppm fluoride dentifrice. Baysan[9] concluded that toothpaste containing 5000 ppm fluoride was significantly better at remineralizing primary root caries lesions than that containing 1100 ppm fluoride. In addition, Ekstrand[10] states that the use of 5000 ppm fluoride toothpaste significantly reduces the amount of accumulated biofilm, the number of S*treptococus muttans* and *Lactobacilus*. Interestingly, it also promotes more calcium fluoride deposits than the traditional fluoride-containing toothpaste. The same group of authors reaffirm in another study that the 5000 ppm fluoride toothpaste is significantly more effective in controlling root caries progression and promoting remineralization compared with the 1450 ppm fluoride toothpaste.[11] Heijnsbroek *et al.*[12] corroborates that additional fluoride seems to be a preventive and therapeutic treatment for root caries. On the other hand, studies by Fernández *et al.*[13] showed that the combination of acidulated phosphate fluoride combined with 1100 ppm dentifrice is as effective as that of 5000 ppm in inhibiting enamel demineralization and remineralizing root lesions.

Considering that the objective of this study was to evaluate the effect of 5000 ppm fluoride dentifrice on the enamel demineralization process inhibition, the results show that it presented lower surface hardness loss when compared to the other groups: control and placebo, a result also observed by Bizhang,[6] who found that there was less mineral loss in the demineralization process of human dentin in the use of 5000 ppm fluoridated dentifrice than in the 250 ppm fluoridated mouthwash. Another similar result was published by Al-Mulla[14] in an *in situ* study aiming to compare the effects on enamel demineralization and fluoride retention in two brushing regimens. They observed that the high-fluoride dentifrice has better anti-caries potential and better fluoride retention in the oral cavity when compared to the 1450 ppm fluoridated dentifrice.

Nordstrom and Birkhed,[4] in an *in situ* study of caries-active adolescents, found that young people who used the 5000ppm fluoridated toothpaste had lower incidence and less caries progression than those who used the 1450 ppm fluoridated toothpaste.

Although there is general agreement among researchers about the importance of fluoride toothpaste as a cheap and easily accessible vehicle for the reduction of dental caries, is still limited the number of studies verifying the recommended methods of fluoride use for individuals with high levels of caries risk and test the effectiveness of high fluoride concentration in the dental demineralization and remineralization process.

Based on the divergence of methodologies of the indicated studies and the need for more evidence on the effectiveness of high-fluoride toothpastes, it is extremely important to keep on searching with the aim of providing to the literature new data that test the efficacy of dentifrices with high concentration of fluoride in the dental demineralization process.

## Conclusion

According to the results, we can conclude that the high-fluoride dentifrice and the conventional dentifrice are able to inhibit the enamel demineralization process, with the test group showing a statistically superior result than the control group, even though further research are needed on this subject.

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## Mini Curriculum and Author's Contribution

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Submitted: 03/27/2020 / Accepted for publication: 03/29/2020

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