

# Comparison of Nano-Hydroxyapatite and Sodium Fluoride Mouthrinse for Remineralization of Incipient Carious Lesions

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## Abstract

**Objective:** Dental caries is an infectious disease that can be prevented in several ways. The aim of this study was to compare the efficacy of sodium fluoride mouthrinse and nano-hydroxyapatite (nano-HA) for the remineralization of incipient caries.

**Materials and Methods:** After obtaining different concentrations of nano-HA (0.2-5-10%), 60 sound premolars fixed in acrylic blocks were coated with nail polish except for one surface. Ten teeth (control group) were stored in distilled water and the remaining 50 samples were demineralized by immersion in 13 ml of 0.1 M lactic acid and 0.2% poly acrylic acid for 48 hours. Their microhardness was then measured and compared to that of the control group. Next, the 50 test teeth were randomly divided into 5 groups of group 1 (negative), group 2 (2% nano-HA), group 3 (5% nano-HA), group 4 (10% nano-HA) and group 5 (0.2 NAF mouthrinse). The microhardness of the teeth was measured after 12 hours of immersion in the above-mentioned solutions. Data were analyzed using repeated measures analysis of variance (ANOVA).

**Results:** Microhardness of all samples decreased significantly after immersion in the demineralization solution and increased following immersion in nano-HA and NAF mouthrinses; however, this increase was not statistically significant ( $P=0.711$ ).

**Conclusion:** Nano-HA and NAF mouthrinses can greatly enhance remineralization and increase tooth microhardness.

**Key words:** Nano-hydroxyapatite; Tooth Remineralization; Sodium Fluoride

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## INTRODUCTION

Dental caries is an infectious disease and its initiation and progression depend on several factors. In a balanced oral environment, saliva contributes to tooth remineralization by supplying mineral components necessary to form

HA. The critical pH for enamel dissolution is about 5.5 [1]. Although several methods such as local/systemic fluoride therapy, diet control and use of fissure sealants are used to prevent dental caries, they are not efficient enough to completely prevent tooth decay [2].

Hydroxyapatite is an important biomaterial and a major component of the mineralized structure of the teeth and bones [3-5]. It is also an important bioceramic for medical and dental applications (including dental implants, orthopedics, alveolar reconstruction and drug delivery systems) due to its biocompatibility and biological and chemical similarity to the bone structure [6].

HA can be used for bone, cementum and artificial root formation and can induce tooth remineralization. With the development of nanotechnology, nano-HA particles are extensively studied in the fields of biomaterials and medicine.

Compared to typical HA, nano-HA has some unique properties such as higher solubility, higher surface energy and optimal biocompatibility. Substituting bone and tooth with have several advantages including high toughness, high strength, high density, long shelf life and optimal biocompatibility [7].

Moreover, it has been reported that nano-HA particles have superior bioactivity compared to larger crystals [8].

In spite of the reports of HA application in various fields of medicine, only a few studies have evaluated the relationship between nano-HA and dental caries.

Results of a study comparing the remineralization effect of combined nano-HA and sodium-fluoride (NaF) on primary carious lesions showed that the greater the amount of nano-HA in NaF mouthrinse, the higher the remineralization of enamel. They suggested a synergistic role for nano-HA combined with a fluoridated mouthrinse [2].

Another study evaluated the effect of nano-HA solution on erosive lesions and showed that enamel microhardness (decreased due to erosive lesion) enhanced significantly after exposure to nano-HA solution [9].

The purpose of this study was to compare the remineralization effect of nano-HA and NaF mouthrinses on the initial carious lesions.

## MATERIALS AND METHODS

In this experimental study, nano-HA (Nanoshel Co., USA) was added to distilled water in predetermined weight percentages (0, 2, 5, 10%) in order to prepare nano-HA mouthrinses of different concentrations.

Sound premolars extracted for orthodontic purposes were collected and fixed in acrylic blocks. After cleaning and polishing the teeth, all surfaces were coated with nail polish, except for one surface. Ten teeth were randomly selected as controls and stored in distilled water during the study. Eventually, Vickers hardness number (VHN) of all samples was measured using Vickers microhardness tester (Shimadzu M g5037, Japan).

In order to develop artificial caries, the samples were immersed in 13 ml of 0.1 M lactic acid and 0.2 M poly acrylic acid with a pH of 5. After demineralization, VHN was measured by applying 200gr force. Then, the samples were randomly divided into 5 groups (one group was exposed to NaF mouthrinse and the remaining 4 to nano-HA mouthrinses of different concentrations). For the initiation of remineralization, all teeth were immersed in artificial saliva for 12 hours [10] before the microhardness testing. Then, 4 groups were prepared as follows:

Group 1: 10 teeth were immersed in distilled water (negative control group) for 12 hours.

Group 2: 10 teeth were immersed in distilled water plus 2% nano-HA for 12 hours.

Group 3: 10 teeth were immersed in distilled water plus 5% nano-HA for 12 hours.

Group 4: 10 teeth were immersed in distilled water plus 10% nano-HA for 12 hours.

Group 5: 10 teeth were immersed in 0.2 % NaF mouthrinse (Behsa Co.) for 12 hours.

Finally, the difference in VHN was calculated in each group and data were analyzed using repeated measures ANOVA. P-values less than 0.05 were considered significant. Statistical analysis was done using SPSS 20 for windows (Chicago, IL, USA)

## RESULTS

The mean baseline microhardness in the 5 groups (control, 2% nano-HA, 5% nano-HA, 10% nano-HA and NaF mouthrinse) was 349.5, 351.5, 373, 379.1 and 369.2 Gpa, respectively and it decreased to 86.7, 65.8, 74.2, 119.4 and 127.8 after immersion in cariogenic solution, respectively. After demineralization, the teeth were immersed in distilled water, NaF and different concentrations of nano-HA mouthrinses. The microhardness of each group was as follows:

Group 1= 60.9, group 2= 78, group 3= 81, group 4= 199 and group 5= 128.1. Repeated measures ANOVA showed that the microhardness values decreased in the test groups (repeated factor P value <0.001) but the magnitude of reduction was not significantly different between the groups (interaction P value=0.615).

The before and after microhardness values were not significantly different between the groups either (between subject P value=0.142). Table 1 shows the mean microhardness of each group before the induction of caries, after the induction of caries and after immersion in the mouthrinses.

Figure 1 shows the mean microhardness of each group, before the induction of caries (1), after the induction of caries (2) and after immersion in the mouthrinses (3).

## DISCUSSION

Dental caries is the most common chronic disease in childhood. It develops as the result of the function of cariogenic microorganisms on fermentable carbohydrates.

Tooth decay can be prevented by the elimination of etiologic factors. Saliva can greatly help in this respect [1].

Use of nano-HA mouthrinse is one way to prevent dental caries. Nano-HA is a calcium-phosphate compound with a similar structure to that of the mineralized part of dentin and enamel and has the potential to remineralize initial carious lesions [11]. It is bioactive and biocompatible [12]. Our study results revealed no significant difference between the effect of various concentrations of nano-HA and NaF mouthrinses on initial carious lesions. Huang examined the effect of Galla Chinensis combined with nano-HA on the remineralization of primary lesions and showed that the increase in enamel microhardness following the application of these materials was greater than distilled water [13]; which confirms our results. However, we compared the effect of different concentrations of nano-HA on the remineralization of initial lesions in human teeth.

Huang evaluated the remineralization effect of nano-HA on animal teeth by means of measuring the surface and cross-sectional microhardness by polarized light microscopy.

**Table 1.** The mean microhardness of groups before the induction of caries, after the induction of caries and after immersion in the mouthrinses

Group		VHN1	VHN2	VHN3
1	Mean	349.50	86.70	88.70
	Std. Deviation	36.939	102.557	128.209
2	Mean	379.10	74.20	97.10
	Std. Deviation	31.543	88.473	107.075
3	Mean	374.00	119.44	124.20
	Std. Deviation	59.703	104.703	110.811
4	Mean	369.20	127.80	128.40
	Std. Deviation	63.894	128.156	119.502
5	Mean	351.50	65.80	77.78
	Std. Deviation	42.735	71.456	99.516
Total	Mean	364.66	94.29	103.76
	Std. Deviation	48.168	99.607	110.720

The results showed that nano-HA enhanced mineral apposition of surface enamel and was able to repair enamel lesions [14]. We also concluded that remineralization of primary enamel lesions increased with the use of different nano-HA concentrations. The results of a study conducted by Min demonstrated that erosive enamel lesions were repaired effectively by the use of 0.25% nano-HA [15]; these findings are in accordance with our results. However, Min used cone-focal laser scanning and scanning electron microscopy on animal teeth.

Mensinkai and Mathews assessed the effect of different types of fluoride mouthrinses on erosive lesions.

The results of both studies revealed that fluoride mouthrinses were advantageous for lesion remineralization; which is consistent with our study results [16, 17].

Haghgoo studied the remineralization of erosive lesions using nano-HA. The results demonstrated that the microhardness of demineralized enamel increased following the application of nano-HA [9]. It is in agreement with our result but we compared different nano-HA concentrations with NaF mouthrinse.

Kim compared the remineralization effect of nano-HA combined with NaF mouthrinse and concluded that nano-HA has a synergetic role in dental remineralization [2]; this result is consistent with ours. Huang investigated the effects of nano-HA on the remineralization of initial enamel lesions and found that nano-HA has a remineralization potential for initial enamel lesions and this finding is similar to our results [18]. However, we studied the remineralization potential of nano-HA mouthrinse on human enamel using VHN and this is the main difference between our study and Huang's investigation. In our study, human teeth were used as samples, but one limitation was to collect premolar teeth only.

We suggest further studies to determine the effect of these solutions on lesion depth and various parts of lesions.

## CONCLUSION

This study showed that both nano-HA and NaF mouthrinses can greatly enhance remineralization and increase tooth microhardness.

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