

Effects of Homemade Nano-Hydroxyapatite and Olive Oil Paste on Remineralization of Early Caries Lesions

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Abstract: Nano-hydroxyapatite (nHAP) particles are a new generation of materials reported to remineralize enamel lesions. The purpose we aimed was to compare the in vitro effects of fluoride gel, sodium fluoride toothpaste, and homemade nHAP paste on remineralizing artificial early enamel caries. Methods: Incipient caries were induced in 21 extracted, sound premolar teeth by storing each specimen in a demineralization solution for 72 hours, followed by pH cycling. The samples (n= 7, each) were then treated with 2% neutral fluoride gel, 0.25% sodium fluoride toothpaste, or homemade nHAP paste, comprising a mixture of nHAP powder and olive oil. After demineralization and remineralization, the results were compared using the DIAGNOdent pen (KaVo, Germany). The data were statistically analyzed using paired t-tests and a one-way ANOVA test. Outcomes: The degree of demineralization in each of the three groups (fluoride gel group, 15.71; sodium fluoride dentifrice group, 15.28; nHAP paste group, 16.71) was significantly elevated compared to baseline (3, 2.5, 2.28, respectively); however, no significant difference was observed between the remineralization readings in each of the three groups (6, 7, 5.5, respectively) ($p > 0.05$). In conclusion, we concluded that the homemade nHAP paste had a beneficial effect on the remineralization of initial enamel caries lesions.

Keywords: Demineralization; dentifrice; remineralization; artificial caries; nano-hydroxyapatite paste

INTRODUCTION

Preventive dentistry is the preferred research area for developments in maintaining natural tooth structure and decreasing the risk of caries¹. Demineralization occurs when the pH of the oral environment falls below 5.5, resulting in the loss of minerals from the tooth surface. In the early stages of enamel decay, remineralization controls the decay process and reverses caries by depositing minerals on the demineralized tooth surface. Calcium and phosphate ions in the saliva can be deposited on the surface of the lesion, which remineralizes the initial lesions².

Remineralization can be achieved with agents such as fluoride, which is administered through gels, toothpaste, and mouthwashes^{3,4}. However, it has been reported that chronic exposure to low fluoride levels can have toxic effects on systemic health⁵. In addition, the extensive use of fluoride, mainly in the form of dentifrices, has contributed to a rising incidence of dental fluorosis⁶.

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Hence, research has emphasized using biomimetic oral care products containing hydroxyapatite particles in preventive dentistry^{7,8}. Nano-hydroxyapatite (nHAP) has the same chemical and physical properties as the enamel apatite structure, shows a high affinity for dental hard tissues⁷, and can penetrate the enamel pores. Therefore, nHAP may serve as a safe and effective alternative dentifrice for home use by patients with limited access to professional dental care.

The ingredients in commercial toothpastes, including abrasives, detergents, and antibacterial agents, have some side effects. Moreover, water in these products can increase the potential for microorganism growth. These limitations have prompted individuals to seek natural alternatives, such as oil-based homemade oral care products, which remove plaque through mechanical action⁹. However, while homemade pastes are popular and contain natural ingredients, they are not necessarily effective. Researchers and dental professionals must also measure the remineralizing properties of homemade pastes to determine their clinical merit.

It is essential to diagnose carious lesions correctly to determine the degree of remineralization within the dental tissue. The DIAGNOdent pen has been used to detect caries using laser fluorescence and measure lesions numerically¹⁰. The DIAGNOdent has been implemented in clinical practice and used effectively since it first entered the market.

With the advent of new remineralizing formulas, more in-vitro research is required to develop alternative homemade dentifrices containing hydroxyapatite particles. This study aimed to determine the effectiveness of nHAP on the remineralization by comparing the in vitro activity of three different remineralizing agents (fluoride gel, sodium fluoride toothpaste, and homemade nHAP and olive oil paste). We hypothesized that the nHAP-based homemade dentifrice would promote equal enamel remineralization compared to fluoride dentifrice and fluoride gel.

MATERIALS AND METHODS

Sample selection and preparation

Twenty-one premolars extracted for orthodontic and periodontal purposes were selected from a collection of teeth. The ethics committee approved this project of Istanbul University Faculty of Dentistry (2018/102). Gingival tissues were manually removed using hand instruments. First, the roots of the teeth were embedded in wax. A 3 × 3-mm² square tape was attached to the buccal enamel surfaces of the samples in all three groups, and the remaining areas were covered with two coats of acid-resistant nail varnish (Max Factor, France). The adhesive tape was then removed from the specimens, and they were kept at room temperature for 24 hours to allow the varnish to dry. The residues were cleaned with ethyl alcohol, and an area of 3 × 3 mm² was obtained for experimental applications. This case isolated the demineralization and remineralization to the window area.

Preparation of demineralizing solution

High-grade analytical chemicals and deionized water were used to prepare the demineralizing solution. Demineralizing solutions were produced using a mixture of 2.2 mM calcium chloride dihydrate, 2.2 mM sodium hydrogen phosphate dihydrate, and 0.05 M lactic acid solution (2H₂O, CaCl₂ = 2.2 mM; 2H₂O, NaH₂PO₄ = 2.2 mM; lactic acid = 0.05 M) at pH 4.5¹¹.

Lesion Formation

All the teeth used in the study were immersed in the demineralizing solution for 72 hours at 37°C, to produce artificial incipient caries lesions.

All the lesions were measured and the scores showed values between 12–24, which indicated the necessity for a preventive therapy, according to the DIAGNOdent pen clinical guideline.

Remineralization solution

The 21 teeth were then maintained in sterilized saliva¹² for one day. Saliva was freshly collected in the morning from a single male donor who chewed paraffin for 5 minutes after rinsing his mouth.

Treatment

The specimens were randomly divided into three groups (n = 7) (Table 1).

Group 1: Fluoride gel (2% Neutral Sodium Fluoride, Imicryl) was applied for 4 minutes to the tooth surfaces according to the manufacturer's instructions.

Group 2: Sodium fluoride toothpaste (Opalescence Whitening Toothpaste, Ultradent) was applied for 2 minutes to the buccal enamel surface. The toothpaste slurry was prepared in a 3:1 weight ratio of deionized water to toothpaste by EN ISO 11609–2017 (Dentistry- Dentifrices - Requirements, test methods, and marking).

Group 3: Homemade nHAP paste (Geistlich, Bio-Oss powder + olive oil) was applied for 2 minutes on the tooth surfaces. The paste was prepared in a 1:1 weight ratio of olive oil to nHAP powder (Geistlich, Bio-Oss) without any fluorides.

DIAGNOdent Pen measurements

The same DIAGNOdent pen was used throughout the study, and one examiner performed all measurements. After calibration with the ceramic standard, the fluorescence of a solid spot on the tooth's smooth surface was measured to provide a baseline value, as per the manufacturer's instructions. DIAGNOdent pen readings were taken at baseline, after lesion formation, after sterilized saliva exposure, and immediately after remineralization. The enamel surface was dried before each measurement, and the device probe (tip 2) was used for smooth scanning surfaces. Each area was scanned twice with the pen, and the highest value from the two readings was registered.

Statistical analysis

The data analysis were performed using the Statistical Package for Social Sciences (SPSS, ver 11, SPSS Inc. Chicago, Illinois, USA). Descriptive statistics for each group were presented as mean and standard deviations. The differences between the baseline DIAGNOdent pen readings and those after demineralization were compared using paired t-tests. The level of significance was set at $p < 0.05$. The differences between the DIAGNOdent pen values of the treatment groups were compared using the one-way ANOVA test ($p < 0.05$).

RESULTS AND DISCUSSION

The readings at baseline, after lesion formation, after sterilized saliva exposure, and after treatment using the DIAGNOdent pen are shown in Table 2. Alterations on the lesion surface due to the demineralization and remineralization process was observed in all enamel specimens. The DIAGNOdent scores after demineralization were significantly elevated compared to those at baseline ($p < 0.05$); however, no significant differences

were observed between the DIAGNOdent scores after remineralization treatment in each of the three groups (fluoride gel group, 6; sodium fluoride dentifrice, 7; nHAP paste group, 5.5) ($p > 0.05$).

Table 1. Ingredients of Remineralizing Agents Used in This Study

Remineralizing agent	Ingredients	Manufacturers
Polimo Fluoride Gel	2% Neutral Sodium Fluoride (9050 ppm)	Imicryl, Konya, TURKEY
Opalescence Whitening Toothpaste (Sodium fluoride dentifrice)	Sodium Fluoride 0.25 % w/w (1100 ppm) Glycerin, Water (aqua), Silica, Sorbitol, Xylitol, Flavor (aroma), Poloxamer, Sodium Lauryl Sulfate, Carbomer, FD&C Blue#1 (CI 42090), FD&C Yellow#5 (CI 19140), Sodium Benzoate, Sodium Hydroxide, Sparkle (CI 77019, CI 77891), Sucralose, Xanthan Gum.	Ultradent, South Jordan, UT, USA
Homemade nHAP paste	Bio-Oss powder (nHAP powder from the cancellous parts of bovine bone) + olive oil	Geistlich Pharma North America Inc. Princeton, NJ USA

Table 2. Mean Values and Standard Deviations of the Three Groups Using DIAGNOdent Pen Scores

Tested Configurations		Measurements Scores (Mean (Standard Deviation))			
	Remineralization material Types	Baseline	After demineralization	After sterilized saliva	After remineralization
1	Polimo Fluoride Gel	3.7 (± 1.26)	15.71 (± 4.55)	12.57 (± 2.14)	6 (± 1.97)
2	Opalescence Whitening Toothpaste (Sodium fluoride dentifrice)	2.57 (± 1.51)	15.29 (± 4.27)	12.29 (± 0.75)	7 (± 0.98)
3	Homemade nHAP paste	2.29 (± 2.35)	16.71 (± 5.01)	13.57 (± 3.62)	5.57 (± 0.52)

In vitro systems are usually preferred for caries research as they are less expensive and time-consuming compared to other testing methods. Artificial caries lesions in the enamel are more reproducible and stable than natural caries lesions¹³. A pH-cycling model was used in the present study, and artificial caries lesions were produced using standardized demineralizing solutions.

Even when using bacterial biofilms and saliva, "artificial mouth" systems cannot fully simulate the complex intraoral conditions that lead to caries development. The saliva covers all the surfaces in the mouth and has a protective pH-increasing effect owing to its enzyme and glycoprotein content. To take advantage of the functional properties of pellicle formation, sterilized saliva was used as a remineralizing solution, as shown by White¹⁴ and Casals et al¹⁵.

The present study could have been followed up using scanning electron microscopy (SEM); however, SEM is not suitable for clinical settings. Moreover, SEM is more expensive than the DIAGNOdent pen and requires a specialized laboratory environment. An analysis of available data indicates the utility of the DIAGNOdent pen for reproducible caries detection, with good sensitivity and specificity¹⁶. The DIAGNOdent pen has been used on smooth surface areas in both in situ¹⁷ and in vivo¹⁸ studies. The results indicated that all the three remineralizing agents had a significant remineralization effect on initial caries. The efficacy of ionic fluoride in toothpaste has been confirmed in recent in vitro studies¹⁹⁻²¹ and is supported by in vivo studies²²⁻²⁴. To date, the most effective remineralizing agent is fluoride. However, the mineral nHAP also has a practical reparative effect on dental tissue²⁵.

We confirmed our hypothesis that the nHAP-based homemade dentifrice would promote equal enamel remineralization relative to the fluoride dentifrice and fluoride gel. In the current study, nHAP was applied for only a short time during the pH cycle. Due to the low solubility of pure hydroxyapatite, there are not sufficient Ca^{2+} and PO_4^{3-} ions available to prevent the enamel dissolution and they increase the stability of hydroxyapatite in enamel. However, tiny-sized nHAP crystals (50-1000 nm) have a filling effect on the pores of the demineralized and porous enamel; therefore, nHAP may have precipitated into the surface pores and formed an infrastructure for the mineralization²⁶. This mineral deposition on the surface is comparable to the precipitation of CaF_2 after NaF application. Further, in the present study, we observed that the homemade nHAP paste, sodium fluoride dentifrice, and fluoride gel had similar remineralization effects in treating initial caries.

This finding is supported by prior studies that reported no significant difference between nHAP and other remineralizing agents, including sodium fluoride²⁷⁻²⁹. In a previous study, nHAP was observed to induce remineralization by forming a homogeneous apatite layer on the demineralized surfaces of enamel after only 10 minutes of treatment. This surface comprises synthetic hydroxyapatite nanocrystals that chemically bond to natural enamel crystals. Additionally, the hardness and elastic modulus of the restored enamel is similar to those of the natural structure⁸. According to transmission electron microscopy (TEM) observations, the nHAP solution is reported to fully integrate into the enamel³⁰.

In the present study, the effects of the fluoride dentifrice and neutral sodium fluoride gel were similar. However, a previous study demonstrated that the microhardness of enamel was significantly higher when treated with a fluoride dentifrice than a neutral

sodium fluoride gel³¹. Increasing fluoride concentrations may not produce a further significant increase in remineralization³². Moreover, some in vitro studies have reported significantly greater remineralization efficiency with 1000 ppm and 1400 ppm sodium fluoride than nHAP treatments^{33,34}. These conflicting results may be attributed to the different variables within the studies (in vitro/in vivo study, type of remineralization agent, application time, etc.).

An in vivo study compared the effectiveness of three different remineralizing agents using the DIAGNOdent pen, in which nHAP was shown to be the most effective one³⁵. Further, a randomized clinical trial has reported that an nHAP-containing toothpaste performed better than a fluoride-containing one, based on DIAGNOdent readings³⁶. In the current study, the nHAP paste was prepared by mixing olive oil with Geistlich Bio-Oss pure nHAP powder with low solubility, making this method easy to use at home and applying to the tooth surface. Hydroxyapatite powder is soluble in olive oil and the food-grade olive oil is harmless to human health. Olive oil has been used in preparations of hydroxyapatite^{37,38} and as an additive agent to hydroxyapatite³⁹. Commercial toothpastes often contain water as an ingredient; however, in a homemade toothpaste, water provides a suitable environment for the growth and proliferation of microorganisms. Therefore, oil is preferred instead of the water.

Quality and safety of biomaterial manufacturing processes are subject to quality management systems, and Geistlich Pharma is certified according to ISO 9001 and ISO 13485 international standards⁴⁰. The pure nHAP particles in the toothpaste formulation increase its overall cost. However, different manufacturing methods may help overcome this economic restriction.

Homemade nHAP dentifrices may be recommended to manage patients with high caries risk and high caries activity. Individuals who do not wish to use fluoride due to toxicity concerns should not neglect their oral health. In these cases, the nHAP may be offered to patients as an alternative to fluoride. Further in vitro studies, controlled in vivo studies, and clinical trials are needed to ascertain the true clinical efficacy of homemade dentifrices containing nHAP.

CONCLUSION

The present study concluded that the homemade nHAP paste had a beneficial effect on the remineralization of initial enamel caries lesions. nHAP may be provided as an alternative to high caries risk patients and for patients with limited access to professional dental care.

CONFLICT OF INTEREST

The author report no conflicts of interest and no funding resources in this study.

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