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Effect of Nano-hydroxyapatite Toothpaste on Microhardness of Primary Teeth Enamel in Comparison with Two other Toothpastes "An in Vitro Study"

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Aim: The purpose of the current study was to investigate the effect of a nano-hydroxyapatite toothpaste on microhardness primary teeth enamel in comparison with two other kinds of toothpaste (fluoride and miswak) regarding their effect on the microhardness of enamel by the Vickers hardness test and surface topography using scanning electron microscopy (SEM)

Materials and methods: forty extracted primary molars were obtained from the outpatient clinic of the Pediatric Dentistry Department, Faculty of Dentistry, Ain-Shams University. samples were grouped into four main groups according to the type of toothpaste used. Group 1: specimens were brushed with Nano hydroxyapatite toothpaste. Group 2: specimens were brushed with fluoride toothpaste. Group 3: specimens were brushed with Miswak toothpaste. Group 4: specimens were immersed in distilled water; specimens were subjected to a microhardness test (the Vicker test) before and after the remineralization stage. The specimens from the Nano-HA groups, the positive control group, and the negative control group were prepared for SEM examination. These were analyzed using a scanning electron microscope.

Results: The most significant finding was achieved in NHAP, followed by fluoride and then miswak, whereas the value was found to be at its lowest in distilled water.

Conclusion: NHA toothpaste exerted remineralizing effects on the artificial carious lesion.

Keywords: Nanohyadroxyapatite, Miswak, Fluoride, Microhardness

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Introduction

The Primary teeth are extremely important in a child's physical, emotional, and general health. The major functions of primary teeth are speech, eating, nutrition, straighter smiles, and excellent oral health. The process of dental caries is dynamic that occurs when demineralization outweighs remineralization. Tooth demineralization is a chemical process by which minerals are removed from any of the hard matter (enamel, dentine, and cementum).[1] The process of demineralization begins at the demitasse face set up inside the hard tooth towel and may progress into cavitation unless arrested by remineralization Early opinion of nascent lesions can lead to the new rule in preventative dentistry in the form of remineralization.[2] The best mode for caries management is the use of remineralizing products for illustration fluoride, casein phosphopeptide stabilized unformed calcium phosphate (CPP- ACP), sugar substitute, bioactive glass containing calcium sodium phosphosilicate(NovaMin), ozone, herbal compound, laser, hydroxyapatite, and nano-hydroxyapatite. If hydroxyapatite is used to treat an early white spot lesion, tooth remineralization can be anticipated to some degree.[3] In caries prevention NHA is incorporated in toothpastes to provide ions that reduce demineralization and improve remineralization. The nano particles can penetrate tooth porosities and they can produce a protective layer on the tooth' s surface. Nanotechnology is gaining interest in cariology and prevention, where NHA is used to remineralize the affected dentin and enamel by the carious attack. In early-stage caries, the hard tissue loses mineral ions by an acid attack coming from bacterial metabolism but the collagen network remains unaffected. The attempt of remineralizing this organic scaffold is materialized by using nanoparticles (NHA, bioactive glass) which act either as a direct replacement of last minerals or as a carrier for lost ions in the carious attack.[4]

Materials and methods

The materials that were used for that study were:

1) Nano hydroxyapatite toothpaste Apagard Apa-kids toothpaste, SANGAI CO.LTD, JPAN
2) Fluoride toothpaste. Signal Unilever, Mashreq personal care company, Egypt.
3) Miswak toothpaste. Dabur miswak, Dabur limited, Egypt.
4) Demineralizing agent. Prepared in lab.
5) Artificial saliva. Prepared in lab.

Study methodology:

1-The sample size estimation:

It was estimated using Epicalc program version 1.02 assuming a power of 80% and alpha = 0.05. The minimal required total sample size was found forty primary molars were randomly divided into four groups (i.e. 10 samples per each group).

2-Sample selection

Cross-section size of forty primary teeth were calculated from the inpatient hospital of the Pediatric Dentistry Department, Faculty of Dentistry, Ain Shams University.

3- Sample medication

All teeth were gutted using pumice- water slurry using polishing encounter at low-speed hand piece to remove any debris or math deposited on the tooth face.

4- Demineralizing stage

All set enamel specimens were immersed in demineralization solution which was (2.2 mM $CaCl_2$,2.2 mM NaH_2PO_4 and0.05 M acetic acid PH4.4) and also were irrigated off with 10 ml distilled water for 20 seconds. All crossbeams were air-dried and stored in saline.

5- Remineralization stage

The prepared specimens were randomly divided into four groups:

Group A: n=10 specimens were brushed with NHA toothpaste.

Group B: n=10 specimens were brushed with fluoride toothpaste.

Group C: n=10 specimens were brushed with Miswak toothpaste.

Group D: n= 10 specimens were immersed in distilled water.

6- Dimension of enamel Microhardness

Microhardness was measured using a Vickers testing machine. Three indentations with 100 µm piecemeal were made on the uncoated area of the enamel. Specimens were subordinated to microhardness tests ahead and after the remineralization stage.

7- Scanning electron microscopy (SEM)

After the Surface microhardness analysis (SMH), samples from the Nano-HA groups, the positive control, and the negative control groups were prepared for SEM analysis. These were examined using a scanning electron microscope. For the SEM examination specimens in each group were air-dried. Each specimen was mounted on SEM and then sputter-coated under vacuum with gold by the (Edwards coater). The SEM-EDX test was performed to qualitatively study the morphological changes of enamel surface such as surface roughness, porosities, and cracks following demineralization and surface healing and mineral deposition after remineralization using SEM model Quanta 250 FEG (Field Emission GUN)2 attached with the EDX unit (Energy Dispersive X-ray Analysis) to measure 2 mineral content after demineralization and remineralization, with accelerating voltage 30 K.V, magnification up to 100000 and resolutions for Gun.1n.

8- Statistical analysis

By examining the data distribution and using the Shapiro-Wilk test, numerical data were examined for normality. The data were presented as mean and standard deviation values. They were anatomized using one-way ANOVA and Tukey's post hoc test, and they had a typical distribution. For all tests, the significance level was set at p 0.05. R statistical analysis software interpretation4.1.3 for Windows was used to conduct the statistical analysis.

Results

1) Microhardness results

1)1. Intergroup comparison:

Mean and standard deviation (SD) values of micro-hardness for different groups were presented in the table 1 and figure 1. There was a significant difference between the different groups ($p<0.001$). The highest value was found in NHAP (143.08±43.74), followed by Fluoride (93.81±37.37), then Muswaik (64.83±35.00), while the lowest value was found in distilled water (0.00±0.00). Post hoc pairwise comparisons showed NHAP to have a significantly higher value than other groups ($p<0.001$). In addition, they showed Fluoride and Muswaik to have significantly higher values than distilled water ($p<0.001$).

Table (1): Mean ± standard deviation (SD) of micro-hardness for different groups

Micro-hardness (mean±SD)				p-value
NHAP	Fluoride	Muswaik	Distilled water	
143.08±43.74^{A}	93.81±37.37^{B}	64.83±35.00^{B}	0.00±0.00^{C}	**<0.001***

Means with different superscript letters are statistically significantly different*; significant ($p \le 0.05$)

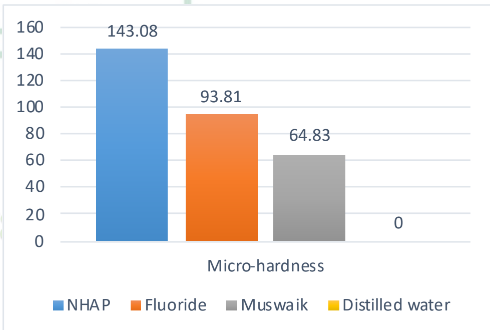

Figure (1): Bar chart showing average micro-hardness in different groups

1)2. Difference from baseline:

A-NHAP:

Mean and standard deviation (SD) values of micro-hardness for baseline and NHAP were presented in table (2) and figure (2) NAHP (322.81±23.21) had a significantly higher value than baseline (179.73±30.19) ($p<0.001$).

Table (2): Mean ± standard deviation (SD) of micro-hardness for baseline and NHAP

Micro-hardness (mean±SD)		p-value
Baseline	NHAP	
179.73±30.19	322.81±23.21	<0.001*

*; significant (p ≤ 0.05)

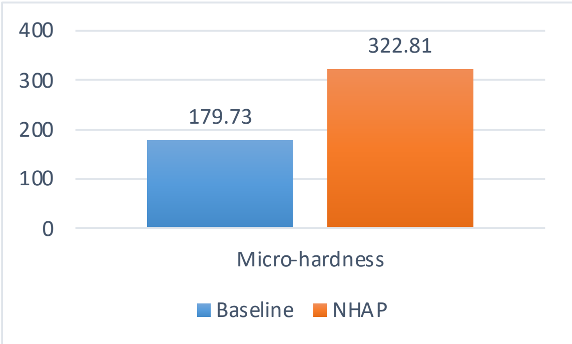

Figure (2): Bar chart showing average micro-hardness for baseline and NHAP

B-Fluoride:

Mean and standard deviation (SD) values of micro-hardness for baseline and fluoride were presented in table (3) and figure (3) Fluoride (273.54±15.26) had significantly higher value than baseline (179.73±30.19) (p<0.001).

Table (3): Mean ± standard deviation (SD) of micro-hardness for baseline and fluoride

Micro-hardness (mean±SD)		p-value
Baseline	Fluoride	
179.73±30.19	273.54±15.26	<0.001*

*; significant (p ≤ 0.05)

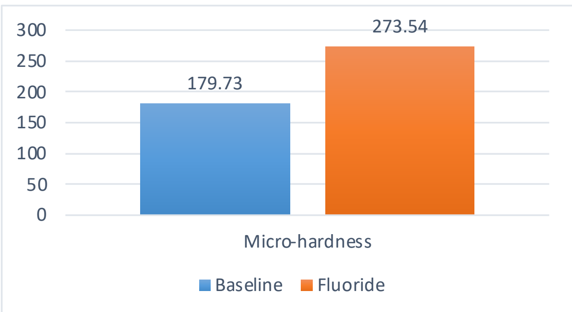

Figure (3): Bar chart showing average micro-hardness for baseline and fluoride

C-Muswaik:

Mean and standard deviation (SD) values of micro-hardness for baseline and muswaik were presented in table (4) and figure (4) Miswaak (244.56±22.45) had a significantly higher value than baseline (179.73±30.19) (p<0.001).

Table (4): Mean ± standard deviation (SD) of micro-hardness for baseline and muswaik

Micro-hardness (mean±SD)		p-value
Baseline	Muswaik	
179.73±30.19	244.56±22.45	<0.001*

*; significant (p ≤ 0.05)

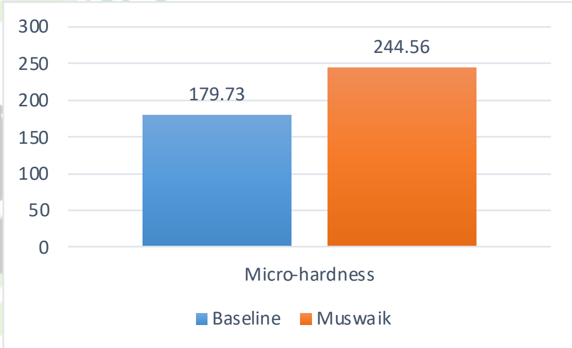

Figure (4): Bar chart showing average micro-hardness for baseline and muswaik

2)Scan Electron -microscope analysis:

2)1. Before and after demineralization Figure (5)

Representative samples of the SEM interpretation are presented as :

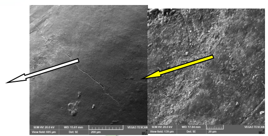

Figure (5): White arow presents the original surface with an intact enamel surface and a yellow arrow in another SEM presents a surface after demineralization since the surfaces become more roughness.

2)2.SEM after brushing with toothpastes

A) After brushing with NHAP Figure (6)

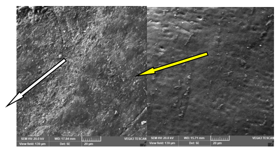

Figure (6):Enamel after brushed with NHAP toothpaste visualized under SEM the surface is smoothed down White arrow present surface after demineralization since the surfaces become more roughness and yellow arrow present enamel brushed with NHP with smoothand homogenous surfaceand no porosity.

B) After brushing with fluoride Figure (7)

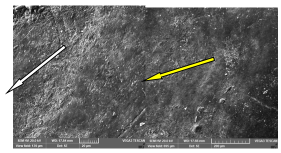

Figure (7): Enamel after brushed with Fluoride toothpaste visualized under SEE the surface is partially smoothed down yellow arrow presented enamel surface brushed with fluoride toothpaste with smooth surface with less porosity.

C) After brushing with miswak Figure (8)

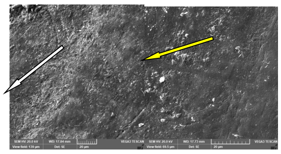

Figure (8):Enamel after brushed with Miswak toothpaste visualized under SEM yellow arrow presented enamel brushed with miswak with less smooth surface and less porosity

Discussion

Dental caries is an infectious microbiological disease that results in destruction of calcified tissues. Caries is a dynamic process which occurs when demineralization exceeds remineralization. However, progression of dental caries is a slow process and during early stages non-invasive intervention can convert the lesion to inactive state from an active state. Early diagnosis of incipient carious lesions has led to a new transaction in preventive dentistry in the form of remineralization. The ultimate treatment modality for caries management is the use of remineralizing products.[5]

The present study was designed to evaluate and compare between remineralization of nano hydroxy appetite, fluoride and miswak toothpastes on enamel primary molars by measuring microhardness of enamel before and after remineralization and evaluate the surface roughness of enamel by the SEM before and after remineralization.[2] Vickers microhardness testing was used in the current investigation because it can be used to test small specimens accurately while being more sensitive to measurement mistakes and less responsive to surface conditions.[6]

In this study forty extracted primary molars were collected free from caries, fracture, hypocalcification, enamel hypoplasia and extrinsic or intrinsic staning. All primary molar teeth were cleaned by using pumice-water slurry using polishing brush at a low-speed hand piece to remove debris or calculus on tooth surface and stored in distilled water before using to prevent dehydration.[7]

Teeth roots were cut, and enamel specimens were obtained and soaked for 10 minutes in 50% ethanol to remove any debris and then were air dried. In this study demineralization solution was used for artificial initial caries formation. all prepared enamel specimens were immersed in demineralization solution at room temperature for 12 hrs. And then were rinsed off with 10ml distilled water for 20 sec and then were dried and stored in saline.[8]

During an acidic attack, chemical dissolution of both the organic and inorganic matrix components takes place. This is brought about by the water content of enamel, which facilitate acid diffusion in and mineral content out of tooth. Specimens then treated by NHA, fluoride and Miswak toothpastes.[9]

The SEM showed that demineralized enamel is characterized by both amorphous and prismatic hydroxy apatite and by an irregular surface. Using demineralization solution cause an exposure of prism structure and loss of both interprismatic and prismatic substance. The SEM micrographs indicated that the NHA toothpaste was capable of forming a homogenous surface of the apatite layer.[10]

The results of this study showed the highest value of SMH was found in NHA followed by Fluoride then Miswak. The specimens treated with NHAT exhibited surface microhardness ratio close to that of the enamel indicating an appetite coating deposition on the demineralized enamel surface.[11]

For SEM analysis, NHAT showed smooth, homogeneous surface with no porosity, while MT and FT showed less surface roughness and slight porosity. This might be due to the characteristics of nanohydroxyapatite, with similarity in enamel structure that can create the induction of initial caries remineralization upon generating a homogeneous apatite complex structure on the demineralized enamel once the toothpaste is applied.[12]

In agreement with this study, another study results suggested additive benefits of nano-HAP incorporation into fluoridated dentifrice on carious lesion surfaces. Compared to the standard fluoride control, the nano-HAP fluoride dentifrice produced directional increase in surface hardness and statistically significant decrease in surface roughness.[13]

Another study found that NHA has higher remineralization effect on enamel of primary teeth than fluoride and Miswak and fluoride has higher reminerization in comparison with Miswak. This disagree with our study since they confirmed that nano hydroxyapatite toothpaste is equivalent or non-inferior to the fluoride toothpaste with respect to remineralization of initial caries lesions and prevention of carious lesion development.[14]

Limitations of present study that results cannot be generalized as it is in vitro study and difficulty of reproducing the oral environment, biofilm oralflora, salivary component, eating habit and oral hygiene.

Conclusions

The following conclusion could be drawn given the constraints of the current study:

1-When carious enamel was remineralized, NHA and F toothpastes showed greater remineralization capacity than Miswak toothpaste.

2-For the initial carious lesion, NHA and F toothpastes could be viewed as curative and preventive measures.

3- The artificial carious lesion might remineralize with NHA toothpaste.

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