Remineralizing Effect of NovaMin and Nano-hydroxyapatite Toothpastes on Initial Enamel Carious Lesions in Primary Teeth

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ABSTRACT

Background: Modern dentistry aims to treat caries as little invasively as possible while preserving the tooth. To do this, use the tooth's ability to remineralize early caries lesions that aren't cavitated. Changing oral environment, can do this and tilt the scales in favor of remineralization rather than demineralization.

Objective: To examine ability of two commercially toothpastes; one containing NovaMin and other containing HAP-NPs to remineralize artificially depleted enamel surfaces on baby teeth. Vickers microhardness tester used to measure surface microhardness. Surface roughness using SJ-210 surface roughness tester. In comparison to fluoridated toothpaste.

Materials and Methods: Primary anterior teeth from 87removed individuals were allocated into three groups at random (n=29). Group A toothpastes include (NovaMin), Group B toothpastes include NHA, and Group C toothpastes contain fluoride. Before teeth were immersed in a demineralizing solution for 96hours, the baseline surface microhardness (SMH) and surface roughness were assessed using the vickers microhardness tester and the SJ-210 surface roughness tester, respectively. Demineralized sample SMH and roughness were measured after 10days of pH cycling.

Results: Mean SMH was the highest value in NovaMin group (216.35±11.13) followed by fluoride (205.19±33.48), while the lowest value was found in Nano-hydroxyapatite group (203.67±14.14). Mean surface roughness was the highest value in fluoride group (0.98±0.27) followed by NovaMin (0.89±0.21), while the lowest value was found in Nano-hydroxyapatite group (0.88±0.21).

Conclusion: NovaMin or Nano-hydroxyapatite are effective as fluoride in preventing the demineralization of enamel and promoting remineralization. For remineralizing caries-like lesions of primary anterior teeth; NovaMin and Nano-hydroxyapatite toothpastes are efficient.

Keywords: Demineralization, Caries, NovaMin, Remineralization, Nano-hydroxyapatite, Surface microhardness, surface roughness.

INTRODUCTION

Dental caries is a complicated illness that affects people differently depending on their gender, age, and race [1].

In children, it is the most prevalent oral infectious illness. Early stages of tooth decay or demineralization, known as incipient carious lesions, can be stopped, reversed, or develop to cavitation [1]. Incipient carious lesions, also known as smooth surface caries or white spot lesions, are active lesions that are restricted to the enamel [2]. By topically applying various fluoride products, including as mouthwash solutions, toothpaste, gels, and varnish, these types of lesions can be remineralized [2,3].

It has been proposed that enamel remineralization, which has been studied for nearly 100 years, has the potential to be one of the most significant developments in the therapeutic therapy of dental caries [4]. Fluoride is a useful tool for minimising enamel demineralization because it forms a calcium fluoride (CaF_2)-like coating on the demineralized surface, which in turn reduces enamel mineral loss from acid assault [5,6].

Casein phosphopeptide (CPP), nanohydroxyapatite (HAP-NP), and NovaMin have all been suggested as fluoride substitutes in recent years due to their anticariogenic effects [7,8].

In dentistry and orthopaedics, hydroxyapatite (HA) is a calcium phosphate that is frequently utilised for bone regeneration applications[9]. The crystal structure and size of (HAP-NP) are substantially comparable to those of natural hydroxyapatite, which adds to their usage in dental care therapies[10].

Nanohydroxyapatite have recently been incorporated to dental care products like toothpastes and mouthwashes to treat dental sensitivity by blocking the open dentinal tubules that are linked to the pulp or to encourage enamel remineralization by adding calcium and phosphate ions to areas where minerals have dissolved, rebuilding its integrity and gloss [10,11].

As a multi-component inorganic product including elements like calcium, sodium, phosphorus, and silicon, bioactive glass (BAG) is being heralded as a major advancement in remineralization technology [12,13].

AIM OF THE STUDY

This in-vitro study's goal is to compare the effectiveness of BAG containing toothpaste (NovaMin) and HAP-NP toothpaste in remineralizing an artificial incipient enamel carious lesion in primary teeth, as measured by changes in surface microhardness (using a Vickers microhardness tester) and surface roughness (using an SJ-210 surface roughness tester).

MATERIALS AND METHODS

Eighty-seven extracted (n=87), sound upper (n=34), lower (n=53), and primary canines were collected from the outpatient clinic of the Pediatric Dentistry and Dental Public Health Department, Faculty

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of Dentistry, Ain Shams University, and rinsed with distilled water before being used in this in vitro comparative study. The required sample is 29 teeth per group;

- Group (1) Fluoridated toothpaste n=29
- Group (2) NovaMin toothpaste n=29
- Group (3) Nano-hydroxyapatite toothpaste n=29

This was done in accordance with the work of **Haghgoo *et al.*** [(14)].

The extracted teeth were cleaned using distilled water using washing bottle then the remaining roots were removed by slow speed a double coated diamond disc (standard, Russia) [(15)]. Teeth were embedded in acrylic resin molds is in a horizontal direction with the labial enamel surface exposed**)**.

Baseline surface microhardness and roughness were recorded:

Baseline surface microhardness measuring:

Using a Vickers microhardness tester (WilsonVH1102, USA), we determined the teeth's initial SMH by pressing on them with a 100gm (HV0.1) force for 10 seconds. The pressure was applied slowly and without contact, driving the indenter deep into the test specimen. The area of the depression was calculated when the weight was taken off by focusing on it using a magnifying glass[(15)]. To eliminate any potential for error, the average microhardness of the specimen was calculated by measuring the results of three separate indentations.

Baseline surface roughness measuring:

SJ-210 Portable Surface Roughness Tester, (Mitutoyo, Japan) was used to measure the teeth's surface roughness at a starting speed of 0.5 mm/min, with the acrylic block positioned so that the surface to be measured was horizontal. The holder was then slid vertically up until the measuring tip touched the surface of the specimen. To eliminate variability, we used a method that averaged the roughness measured from three separate indentations [(16)].

Producing demineralizing and remineralizing solutions:

Demineralizing and remineralizing solutions were prepared by Department of Biomaterials, Faculty of Pharmacy, Ain Shams University.

Analytical grade chemicals and distilled water were used to create the buffered demineralizing and remineralizing solutions. To achieve a PH of 4.4, 1M of potassium hydroxide was added to the demineralizing solution, which also included 2.2mM of calcium chloride and 2.2mM of sodium phosphate. 1.5mM Calcium Chloride, 0.9mM Sodium Phosphate, 0.15M Potassium Chloride, ph 7.0; these were the components of the remineralizing solution [(17)].

Preparation of artificial carious lesion:

A simulated carious lesion in the enamel was created by soaking samples in a demineralizing solution for 96 hours [(17)].

Samples were then measured for microhardness using a Vickers microhardness tester, (TUKON^{TM} 1102, Germany), by applying100 gm (HV0.1) load for10 seconds and surface roughness using SJ-210 Portable Surface Roughness Tester, (Mitutoyo, Japan) after 96 h of initial demineralization (2nd time).

The pH-cycling process is started during the manufacture of toothpaste using a magnetic mixer on a vibrating machine:

With the aid of a magnetic mixer mounted on a vibrating machine, tooth pastes were made in the form of a solution using a 15 gram paste and 45 ml of distilled water combination (portable magnetic stirrer, Stuart, United Kingdom).

The pH-cycling model (10 days):

For 10 days, the samples were put in the pH-cycling system on a cylindrical beaker. Every cycle includes a three-hour demineralization process twice daily followed by a two-hour immersion in a remineralizing solution. Before and after the first and second demineralizing cycles, samples were treated for one minute with a toothpaste solution made of 3:1 distilled water to toothpaste, and they were then left in a remineralizing solution overnight to simulate fake saliva **(**Figure **1)**. Between steps, samples were washed thoroughly with distilled water using washing bottle (60 seconds) [(17)].

Finally, surface microhardness and roughness was then recorded (3rd time) after remineralization (after ten days of the pH-cycling).

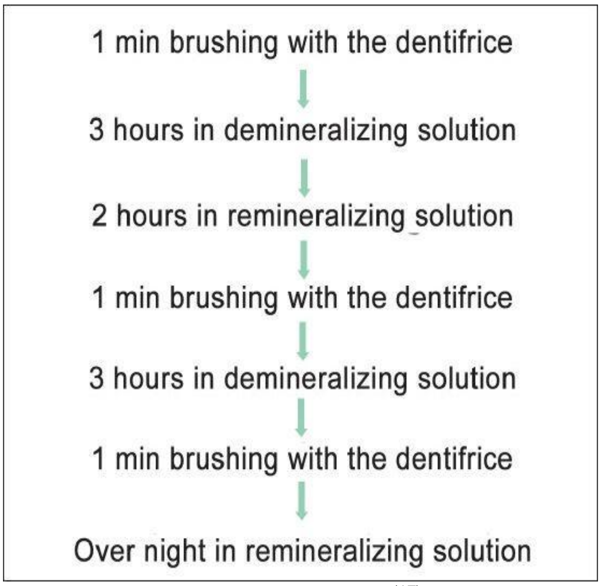

Figure (1): The pH-cycling steps [(17)].

Sample size:

Sample size calculation indicated eighty-seven extracted (n=87), upper and lower primary incisors teeth and primary canine were selected for this in vitro study. The required sample is 29 teeth per group, first group for fluoridated toothpaste, second group for NovaMin toothpaste and third group for HAP-NP toothpaste. This was done in accordance with the work of **Haghgoo *et al.*** [14].

Ethical consent:

This study does not contain any studies with human participants or animals. This study took an exemption from the Scientific Research Ethics Committee, Ain Shams University, (78) 21-2-2018 with number (FDASU-Rec EM021837).

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). P value < 0.05 was considered significant.

RESULTS

Different upper and lowercase superscript letters indicate a statistically significant difference within the same vertical column and horizontal row respectively*; significant ($p \leq 0.05$) (Table **1**).

Table (1): Mean ± S.D for microhardness in different groups

Measurement time	Microhardness (mean±SD)			p-value
	Fluoride	NovaMin	Nano-hydroxyapatite	
Baseline	268.69±23.27	270.53±9.25	269.78±13.03	**0.972**
Demineralization	144.62±23.74	142.96±13.13	141.88±0.89	**0.933**
Remineralization	205.19±33.48	216.35±11.13	203.67±14.14	**0.406**
p-value	**<0.001***	**<0.001***	**<0.001***	

Different upper and lowercase superscript letters indicate a statistically significant difference within the same vertical column and horizontal row respectively*; significant ($p \leq 0.05$) (Table **2**).

Table (2): Mean ± S.D for surface roughness in different groups

Measurement time	Surface roughness (mean±SD)			p-value
	Fluoride	NovaMin	Nano-hydroxyapatite	
Baseline	0.64 ± 0.38^{Ab}	0.68 ± 0.12^{Ac}	0.66 ± 0.20^{Ac}	**0.946**
Demineralization	1.17 ± 0.35^{Aa}	1.19 ± 0.12^{Aa}	1.16 ± 0.12^{Aa}	**0.951**
Remineralization	0.98 ± 0.27^{Aa}	0.89 ± 0.21^{Ab}	0.88 ± 0.21^{Ab}	**0.599**
p-value	**<0.001***	**<0.001***	**<0.001***	

There was a moderate negative correlation between microhardness and surface roughness that was statistically significant ($r = -0.630$, $p<0.001$) (Table **3**).

Table (3): Correlation between microhardness and surface roughness.

Variables	r	p-value
Microhardness	-0.630	**<0.001***
Surface roughness		

r: Pearson's correlation coefficient *; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

DISCUSSION

Despite significant progress in cariology, dental caries continues to be a severe concern for people all over the world. The early stages of a carious lesion in enamel cause the tooth's surface to lose its natural translucency, giving the appearance of a white spot[18,19]. Initial enamel lesions have intact surfaces, but their mineral concentration is minimal in comparison to that of healthy enamel, resulting in a softer surface hardness[19,20].

Considering how important the enamel surface layer is to the progression of caries, the goal of this study was to compare the remineralization potential of two commercially available toothpastes (one with NovaMin and one with HAP-NP) on artificially demineralized enamel surfaces in primary teeth. This was done by measuring the SMH with a Vickers microhardness tester and the surface roughness with an SJ-210 surface roughness tester[21].

Because NovaMin, an amorphous sodium calcium- phosphor silicate, is extremely reactive in water and, as a tiny particle size powder, may physically occlude dentinal tubules, it and HAP-NP containing toothpastes were chosen[22,23]. HAP-NP is an inert chemical that has a strong affinity for dental enamel. Natural enamel HA crystals are very similar in appearance to HAP-NP[10]. No significant side effects have been documented for NovaMin or HAP-NP[24], therefore they are deemed acceptable for preventing caries in both children and adults. However, hypoplastic areas have been described after excessive usage or ingestion of fluoridated toothpastes[24].

Surface microhardness and surface roughness measurements were performed at three experimental times: (1st time) sound enamel (baseline), (2nd time) artificial carious lesion formation and (3rd time) post-treatment (after the application of remineralizing agents).

Due to the relevance of the surface layer in caries development, the assessment of changes in this area is significant, making SMH an appropriate approach for evaluating the deremineralization process, all specimens were tested for both SMH and surface roughness. Materials like enamel, which have a tiny microstructure and are non-homogeneous or prone to breaking, are good candidates for microhardness assessment. In addition, demineralization and remineralization experiments may use SMH indentations since they are quick, easy, and nondestructive[25].

Vickers microhardness tester was used to record SMH (WilsonVH1102, USA). Knoops and Vickers are the two main existing methods for measuring microhardness. Microhardness testing using the Vickers method was used because it is precise even with tiny specimens and is less affected by surface conditions than other methods[26].

SJ-210 Portable Surface Roughness Tester was used to evaluate the surface's roughness (Mitutoyo, Japan). This device has been reported to have high accuracy and measurement speed with numerous innovative features.

There was no statistically significant difference between the various groups when samples were assessed for baseline (sound enamel) SMH and surface roughness for the first time.

The samples were tested for SMH and surface roughness a second time after the artificial carious lesion had developed in the enamel (after 96 hours in the demineralizing solution). All groups' SMH values had decreased, while all groups' surface roughness values had increased.

To imitate a real-world scenario, a modified version of the pH-cycling approach was used in this work [17]. This consisted of two 3 hour demineralizing cycles each day, separated by 2 hour and overnight remineralizing cycles. Additionally, toothpaste was administered three times each day to simulate cleaning teeth in the morning, noon, and before night [27]. The remineralizing solutions employed in the research were identical to those previously used by ten Cate and Duijsters and were designed to mimic supersaturation by apatite minerals prevalent in saliva [27].

Three hours is allotted for demineralization during the pH cycling phase to mimic the potential length of demineralization in the oral cavity [27]. It is important to note that there are many differences between cycle models and in vivo circumstances. The pH-cycling model was not completely accurate in simulating the acidic circumstances seen in the mouth, where the pH levels are determined by an individual's diet, dental hygiene routines, use of fluoride, and the type and composition of saliva and biofilm. For additional verification of the present findings, the remineralizing agents investigated in this work should also be assessed in vivo [27].

A rise in mean microhardness was seen after the prescribed dentifrice treatment regimen. Fluoride group came in second with the greatest value, while the HAP-NP group had the lowest score.

The rapid exchange of sodium ions from the BAG particles with hydrogen cations (in the form of H_3O^+) in the aqueous environment surrounding the tooth, i.e., saliva in the oral cavity, explains the observed release of calcium and phosphate (PO_4) ions from the glass, which is the primary explanation for the observed effects [23]. The initial contact with water triggers a brief, localised increase in pH due to the release of salt. In response to the increase in pH, a calcium phosphate layer forms as a result of precipitation aided by the BAG's elevated calcium and phosphate ions. As these reactions continue, this layer begins to crystallise as hydroxycarbonate apatite (HCA) [23].

In addition, BAG deposits were found on the enamel surface of all the specimens, and it was found that as the reactions progressed and Ca-P compounds were formed, this layer crystallised within the HA, generating a structure that chemically and physically resembled biological apatite. According to these results, BAG deposits may store ions that can be redeployed to areas at risk of demineralization[28]. This may shed light on the reason why NovaMin dentifrice is so much harder than other brands.

The findings of **Alauddin *et al.***[29], which showed that toothpaste containing NovaMin produced more remineralization than fluoride, provide support for the SMH of tooth enamel results in this investigation.

Furthermore, **Rehder Neto *et al.*** [30] demonstrated that the SMH of dental enamel increased following treatment with NovaMin toothpaste, whether used alone or in combination with fluoride. Better remineralization of caries-like lesions in bovine teeth was observed with NovaMin toothpaste, both when used alone and in combination with fluoride, compared to fluoridated toothpastes without NovaMin[30]. Despite the fact that the present research was done on human teeth, its results support theirs.

The SMH of dental enamel rose after treatment with fluoride and a toothpaste containing NovaMin, according the findings of a research by **Golpaygani *et al.***[31]. Their investigation found that NovaMin was much more effective than toothpaste with 1.1% fluoride; these results were similarly consistent with the results of the present study.

The research by **Haghgoo *et al.***[14] also supported these findings, which showed that SMH was greater in the teeth treated with NovaMin toothpaste than in the teeth treated with HAP-NP.

Contrary to **Tschoppe *et al.***[11] findings, which looked at the effects of toothpaste containing HAP-NP on enamel remineralization and discovered that HAP-NP toothpaste had higher efficacy for enamel remineralization than amine fluoride toothpaste when measuring SMH, the results of the present study were in disagreement with those results. There might be reading mistakes in the indentation length, tooth type, and load, as well as improper specimen preparation.

After using the appropriate dentifrices, a reduction in mean surface roughness was seen; the fluoride group had the greatest value, followed by the NovaMin group, and the HAP-NP group had the lowest value.

The study's findings may be explained by the fact that toothpastes containing HAP-NP have a significant affinity for binding to proteins as well as plaque and bacterial pieces. Due to the size of nanoparticles, which significantly raises the surface area that proteins can bind to, this capability is possible. Additionally, HAP-NP fills microscopic gaps and depressions on the surface of the enamel, a function made possible by the small size of the particles that make up the substance[10,11].

This might explain the lowest roughness values recorded for the HAP-NP group dentifrice after remineralization.

The results of surface roughness of tooth enamel were supported by results obtained in the studies by **Selivany and Al-Hano**[32] in which HAP-NP had exhibit a higher ability to reduce the surface roughness after laser bleaching than other tested pastes (NovaMin and kin sense fluoride).

Additionally, our findings supported **Wang *et al.*'s**[33] study, which shown that when surface roughness was measured, BAG toothpastes had a better remineralization efficacy than fluoride toothpaste.

Our findings indicated that there was a large decrease in microhardness values in each group following demineralization, followed by a considerable rise following remineralization, however there were no significant differences between the analysed groups. This would suggest that the three toothpastes have the capacity to remineralize early caries lesions.

Additionally, surface roughness studies demonstrated a large rise following demineralization, followed by a considerable decrease after remineralization. But there were no appreciable variations among the three groups that were examined. This means that the three studied toothpastes have remineralization potential.

It's important to note that during the current investigation, we attempted to simulate the formulation of natural saliva. The generalisation to the clinical context must be done with caution because there are always some discrepancies between natural and artificial saliva. Also, more days in PH cycling model is recommended to provide sufficient time for detecting significant changes in SMH and surface roughness.

CONCLUSION

Both NovaMin or HAP-NP are as effective as fluoride in preventing the demineralization of enamel and promoting remineralization of caries-like lesions in primary anterior teeth, NovaMin gives the highest value for SMH after remineralization, HAP-NP gives the lowest value for the surface roughness after remineralization and there was a negative correlation between microhardness and surface roughness.

RECOMMENDATIONS

Within the limitation of this study, the following can be recommended:

- Conducting in vivo trials to measure the remineralizing potential of the tested materials in natural oral environment.
- More studies are recommended to investigate remineralizing effect of NovaMin or HAP-NP in comparison to other remineralizing agents as fluoride.
- A 15 days PH cycling model is recommended to provide sufficient time for detecting significant changes in surface roughness.

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