SURFACE MICROHARDNESS RETENTION OF PRIMARY TEETH BY TWO DIFFERENT NON-FLUORIDATED BIOACTIVE AGENTS: AN IN-VITRO STUDY

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ORIGINAL RESEARCH

ABSTRACT

BACKGROUND: Alternatives to fluoride as a remineralizing agent have been explored in the field of preventive dentistry to overcome the risk of dental fluorosis and explore materials that can replicate the microstructure of the hydroxyapatite crystals. *AIM:* The aim of this in vitro study is to compare the surface microhardness and thus the remineralizing potential of two non-fluoridated bioactive materials in artificially induced early enamel caries on primary teeth. ***MATERIALS AND METHODS:*** A total of 60 extracted primary molars were divided into three groups: Group I (Novamin; n=20), Group II (n-HAP; n=20), and Group III (control; n=20) and were subjected to a pH cycling model to replicate the intraoral environment of demineralization and remineralization. Surface microhardness measurements were taken at baseline, after demineralization, and after remineralization. ***RESULTS:*** Test of significance using ANOVA did not show any notable difference across all three groups at all three time points. Recovery rate of surface microhardness was highest for NovaMin, followed by n-HAP and control group. ***CONCLUSION:*** NovaMin and n-HAP show promise as remineralizing agents for early enamel caries in primary molars.

Keywords*:* Dental Caries, Tooth demineralization, Tooth remineralization, NovaMin, Nano-hydroxyapatite, Pediatric Dentistry

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INTRODUCTION

Remineralization is the biological process of the oral cavity where the teeth affected by incipient white spot lesions can be reversed back to their microarchitecture through the exchange of minerals in the saliva. An imbalance in the pH of the oral architecture and a subsequent impact of the saliva's secretory rate can lead to further progression of the early lesion towards the irreversible stages.[1]

Fluoride is the most common remineralizing tool used in dentifrices. However, fluoride's inability to replicate the microarchitecture of the natural mineral crystal due to disoriented and disorganized crystallite formation and the risk of it being the harbinger of dental fluorosis has paved the way for researchers to explore better remineralizing agents.[2]

The advent of newer diagnostic aids and the concept of minimal intervention dentistry led researchers to develop synthetic agents that can remineralize the tooth surface whenever a white spot lesion is diagnosed. Two such remineralizing agents are calcium sodium phosphosilicate bioactive glass (trade name: NovaMin) and nano-hydroxyapatite crystals (n-HAP). While NovaMin was first introduced in the late 1960s, n-HAP was first given an approval for its use as an anti-caries agent in 1993.[3,4]

The application of NovaMin to the tooth surface leads to a reaction between the oral fluids where sodium ions of NovaMin exchange with the hydrogen ions in saliva. This leads to the release of calcium and phosphate ions from NovaMin, thereby leading to the formation of a poly-condensed silica-rich gel layer that provides nucleation sites for the precipitation of calcium phosphate. At this stage, the enamel appears as a 'flower-field' at the microscopic level. Eventually, this layer crystallizes into hydroxycarbonate apatite, that prevents demineralization and promotes the uptake of biological ions.[3,5]

n-HAP are favorable for their optical properties, biocompatibility, low solubility, hydrophilic property and greater surface area. The size of the n-HAP crystals is 50-100 nm in length and 20-40 nm in width. The nanocrystals can easily adhere in the pores created due to demineralization, wherein they aggregate and form micro-clusters leading to the formation of a uniform apatite layer.[1,6]

It has been observed that in the deciduous dentition, the critical pH is significantly higher in children as compared to adults in both stimulated and unstimulated saliva. Moreover, children exhibit a greater thermodynamic driving force for demineralization at a low oral pH, and a lower force of remineralization at a normal oral pH. This makes the progression of caries from an incipient lesion to an irreversible stage in deciduous dentition faster as compared to permanent dentition. This is further ascertained by the salivary calcium concentrations which is lower in children compared to adults.[7]

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The objective of this study was to compare the remineralization potential of NovaMin and n-HAP following demineralization (early incipient lesion) in extracted primary teeth. Surface microhardness was used as an indicator to evaluate the efficacy of remineralization. CRIS (Checklist for Reporting of In-vitro Studies) guidelines were followed for the reporting of this study.

MATERIALS AND METHODS

In order to carry out this study, we required sound, caries-free primary molars without any hypoplastic defects, restorations, fractures, or white spot lesions on any surface. Using G-power software, a F-test (ANOVA, fixed-effects, omnibus, one-way) was used where an effect-size of 0.25 at an alpha-error probability of 0.05 and a power of 80% inferred that a sample size of at least 52 would be needed wherein each group must have at least 17.33 samples. Thus, in our study, a total of 60 primary molars were randomly divided into three groups of 20 each: Group I (NovaMin), Group II (n-HAP), and Group III (control). All teeth were kept in 10% formalin before use.

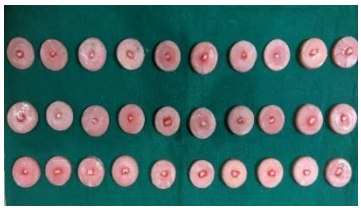

Figure 1 Samples mounted on acrylic

Figure 2 Vickers Microhardness Tester

Teeth were then cleared of soft debris using ultrasonic scaler, rubber cup, and prophylactic paste. The radicular portions of all teeth were sectioned 1 mm above the cemento-enamel junction using abrasive discs. An acid-resistant nail varnish (Revlon, USA) was applied to keep the tooth surface exposed for testing purpose.

Figure 3 Solutions used for formulating the demineralizing solution

All samples were mounted with the help of a stainless steel mould and self-cure acrylic resin (Figure 1). These blocks were stored in artificial saliva (prepared by mixing calcium chloride 1.5 mmol/l, potassium chloride 50 mmol/l, potassium dihydrogen phosphate 0.9 mmol/l, and Tris buffer 20 mmol/l) at 37°C at a pH of 7.4.

Figure 4 NovaMin and n-HAP agents

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A baseline evaluation of surface microhardness was then done using Digital Vickers Micro Hardness Tester (Model: MV1- PC; Fuel Instruments and Engineers Pvt. Ltd.) (Figure 2). A load of 100 grams was applied for 10 seconds on three different areas on the occlusal surface. The average values of the three readings were taken into consideration for each sample.

Demineralization of all samples was done by immersing them in 3 mL of demineralizing solution for 96 hours. This solution was prepared by mixing 3.1 mmol/L calcium chloride, 3.1 mmol/L sodium dihydrogen orthophosphate, and 50 mmol/L glacial acetic acid. 1 mol/L of potassium hydroxide was used to adjust the pH of this solution to 4.5 (Figure 3). The solution was replenished after the first 48 hours. All specimen were then rinsed with 15 mL of deionized water. Another reading of surface microhardness was taken for all samples.

In order to imitate the pH changes of the oral cavity, all samples went through a pH cycling regimen of alternative remineralization and demineralization for 14 days. In the 24-hour cycle, sample were immersed in a demineralizing solution for 3 hours twice a day, followed by 3 minutes of exposure to their respective remineralizing agents.

In Group I, NovaMin was delivered through Vantej® toothpaste whereas in Group II, nano-hydroxyapatite crystals were delivered through Aclaim® toothpaste (Figure 4). The toothpastes were used in a pea-sized amount. Artificial saliva was used as a remineralizing agent. All solutions were replenished after 24 hours and separate containers were kept for each of them. A manual pH electrode meter was used for adjusting and monitoring the pH of the solutions.

The control group did not receive any intervention. After 14 days, the samples were subject to surface microhardness test yet again. The recorded data was then tabulated and sent further for statistical analysis.

RESULTS

Descriptive statistics were used for calculating the mean for all samples in each group at three different time points: baseline, after demineralization (DML), and after remineralization (RML) [Table 1]. Paired t-tests were opted for intragroup comparisons whereas repeated measures ANOVA was carried out for intergroup comparisons using the SPSS 20.0 software.

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Group	Time	Mean	SD	SE
Group I (n=20) Novamin	Baseline	322.6	7.65	1.7
	After DML	178.9	9.88	2.2
	After RML	238.8	10.2	2.3
Group II (n=20) n-HAP	Baseline	345.5	10.1	2.3
	After DML	180.2	10.7	2.4
	After RML	232.2	9.23	2.1
Group III (n=20) Control	Baseline	339.7	9.31	2.1
	After DML	190.8	9.75	2.2
	After RML	207.6	9.5	2.1

Table 1 Descriptive Statistics

Comparison	F-statistic	p-value
Baseline		
Novamin vs n-HAP	1.418	0.247
Novamin vs Control	0.16	0.853
n-HAP vs Control	0.267	0.767
After demineralization		
Novamin vs n-HAP	0.001	0.998
Novamin vs Control	0.069	0.933
n-HAP vs Control	0.051	0.951
After remineralization		
Novamin vs n-HAP	0.259	0.615
Novamin vs Control	1.512	0.236
n-HAP vs Control	0.097	0.907

Table 2 ANOVA Test for Intergroup

Repeated measures ANOVA did not reveal any significant change in the surface microhardness values across all groups at all three time points [Table 2]. However, intragroup comparison of paired t-tests revealed that apart from the change in microhardness values from baseline to after demineralization in Group II, all changes were statistically significant ($p<0.05$) [Table 3].

Group	Comparison	t-statistic	p-value
Novamin	Baseline vs After DML	-24.179	<0.001
Novamin	Baseline vs After RML	-5.138	<0.001
n-HAP	Baseline vs After DML	0.405	0.689
n-HAP	Baseline vs After RML	-5.732	<0.001
Control	Baseline vs After DML	-11.808	<0.001
Control	Baseline vs After RML	-7.866	<0.001

Table 3 Paired t-test for intragroup comparison

The recovery of surface microhardness was calculated using the formula proposed by Joshi et al.[1]:

% Surface Microhardness Recovery = (VHN Remineralization – VHN Demineralization/ VHN Baseline – VHN Demineralization) x 100

Based on this the recovery of surface microhardness was highest in the NovaMin group (41.68%) followed by n-HAP (31.45%) and control group (11.28%).

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DISCUSSION

The concept of using a pH cycling model to understand the effect of demineralization and remineralization on artificially induced enamel caries was first done in 1982.[8] The current study is based on the same principle. A pH modeling cycle reflects the dietary habits, brushing habits, and intraoral habitat in an in vitro environment.[1] The preparation of demineralizing solution and artificial saliva is based on the recommended formulations of Patil et al. and Wang et al. respectively.[9,10]

Sectioning of the radicular portions helped in standardizing the sizes of all specimen. Acrylic served as an ideal material for the stabilization of the samples as it makes the mounted teeth stable under the load applied during microhardness measurement. Vicker's hardness test was preferred over Knoop's hardness test in our study as the shape of the indent of Vicker's hardness test was easy and accurate for the purpose of measurement.[1]

This study demonstrated that compared to the control group, both NovaMin and n-HAP showed a statistically significant difference in terms of remineralization potential. NovaMin had a better surface microhardness compared to n-HAP, but the difference was not statistically significant. This is similar to previous in vitro studies (based on pH cycling model) carried out by Joshi et al. and Haghgoo et al.[1,11] However, the results are contrasting to the study by Manchery et al. wherein the remineralization potential of n-HAP was significantly better compared to NovaMin.[2]

Since the oral cavity is a dynamic and complex system consisting of both stimulated and unstimulated saliva, salivary pellicle, plaque, and other intermittent acid attacks, it is difficult to replicate all these factors in the form of an in vitro simulation. In addition to this limitation, the application of remineralizing agents on vital teeth in long-term clinical trials is a drawback that future research must focus upon. Furthermore, while our study uses surface microhardness as a representative for remineralization efficacy, advanced quantitative measures and advanced qualitative topographical assessments in the form of scanning electron microscopy and polarizing light microscopy can give a more precise idea of remineralization potential of the test agents.

CONCLUSION

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Within the limitations of the present study, it can be concluded that both n-HAP and NovaMin show promise as remineralizing agents when used in primary teeth with early enamel caries.

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