Assessment of The Remineralization Potential of Recently Developed Nano Hydroxyapatite on The Demineralized Enamel Around orthodontic Brackets

Mai Gamal[1], Ahmed A.El-Baily[2], Manal F.Osman[3], Marwa S. Shamaa[4]

[1]Post Graduate, [2,3]Professor, [4]Lecturer, [2,4]Dept. of Orthodontics, [3]Dept. of Biomaterial, Mansoura University, Egypt

***Corresponding Author:**

Email: reham.elashery@yahoo.com

Abstract

Objective: This study was evaluated the ability of Nano hydroxyapatite (nano-HAP) to remineralize the demineralized enamel layer around the orthodontic brackets.

Materials and Methods: This study was done on thirty sound freshly extracted permenant premolars. 3M-Unitek brackets were bonded to the buccal surfaces of all specimens. The specimens were artificially demineralized and randomly classified into 3 equal groups A, B and C. They were immersed in artificial saliva for 10days and during this period, group B was treated with 20% nano-HAP varnish, group C was treated with 3%nano-HAP +0.145%sodium fluoride(NaF) paste, while group A was the control group. Selected specimens of each group were scanned by scanning electron microscope (SEM) and assessed by energy dispersive x-ray analysis(EDAX) before and after remineralization, then all samples were subjected to enamel color changes assessment by spectrophotometer and surface roughness assessment by profilometer. The results were statistically analyzed. Significance of the results was set at 0.05.

Results: Showed that there were significant increase ($P<0.001$) in both Calcium and Phosphorus levels after remineralization by nano-HAP. This was detected by EDAX and confirmed by SEM. There was a significant improvement in the color measurements after application of nano-HAP in the study groups when compared to the control group ($P<0.001$), also the surface roughness has significantly decreased ($P<0.001$).

Conclusion: The findings of our study indicated that nano-HAP with the used protocol in this study could be effective in repairing the demineralized enamel around the orthodontic brackets, restoringthe enamel surface smoothness and its color.

Keywords: Demineralization, Brackets, Remineralization; Nanohydroxyapetite.

Introduction

Enamel demineralization next to orthodontic brackets is a very common and clinically relevant adverse effect of fixed orthodontics. In its early stages, it is represented as chalky or ice like whitish spot lesions.(1) The surface irregularities of orthodontic brackets, bands, wires and other retentive orthodontic components would hinder the naturally occurring self-cleansing mechanisms, such as the movement of oral musculature and saliva. This creates stagnation areas for the acidic bacterial plaque layers, which aggregate around the orthodontic appliance margins, thus making tooth cleaning process is a hard task.(2)

Featherstone et al.,(3) has documented that clinically, formation of white spots around orthodontic attachments may occur in very fast manner, it takes only about 4 weeks during the orthodontic treatment; however it takes about 24 weeks in non-orthodontic patient. Enamel demineralization usually begins with dissolution of the subsurface carbonated hydroxyapetite crystals under the effect of low PH of surrounding acidic media, that's creating fine micro pores& gaps in between the enamel rods.(3) Typically, white spot lesions (WSLs) formed around the brackets were found to be localized on the middle and cervical thirds of the teeth.(4)

Surface roughness is a term often described a closely spaced irregularities or uneven, irregular texture enamel surface.[5] Roughness is usually occurs as a consequence to enamel demineralization, associated loss of surface minerals content and creation of subsurface micro-pores. The affected demineralized areas are characterized by surface roughness, loss of enamel translucency& alteration of the refractive index leading to eminent visual enamel opacity. That's because the demineralized enamel surface could scatter more light than intact one,[6] thus resulting in unaesthetic façades that may require conservative intervention in severe cases. Controlling of these demineralized spots comprises prophylactic routines for averting its appearance and also assisting methods of enhancing remineralization of these existing lesions.

Management of white spot lesions WSLs should be of a preventive rather than curative approach.[7] Besides to regular professional oral hygiene visits, pre-emptive strategies involving oral health promotion, patient education &compliance is required.

Enamel repair occurs through the deposition of depleted minerals in the demineralized defects at a molecular level.[8] So repairing the exhausted enamel surface with theneeded calcium& phosphate ions would be benefits.[9,10] Zhang et al,[11] reported that nano-HAP can afford Ca^{+2} & phosphorous levels that were enough to enhance stability of hydroxyapetite, stop dissolution of dental enamel and permitted the Ca^{+2} &phosphorous to invade the enamel surface continuously to fill the vacant gaps.

Synthetic Nano-hydroxyapatite (nano-HAP**)**

Recent studies considered it as one of the most common biological and bioactive materials.[12,13] The Nano-structuring of nano-HAP affects greatly its biocompatibility by increasing its surface area & decreasing its atomicity as authenticated by Kaehler.[14] The size of nano-HAP crystalsis varying between 50 and 100nm.Many studies assessed the efficacy of nano-HAP as a remineralizing agent on decalcified enamel specimens. They concluded that nano-HAP is more effective under acidic conditions than under neutral conditions. This is a main difference between nano-hydroxyapatite and fluoride because demineralization is enhanced under acidic conditions, but since nano-hydroxyapatite also thrives in an acidic environment, it can prevent mineral loss more effectively than fluoride(Fl).[15]

Peter Tschoppe et al.,[16] reported that nano-HAP pastes revealed higher remineralizing results on bovine enamel samples compared to amine fluoride toothpastes. However, most of previous studies utilized the nano-HAP prepared in low concentrations, not in pure form and limited studies discussed the remineralization potential of pure nano-HAP in high concentration on bovine teeth only. Therefore, the aim of present study was to investigate the remineralization characteristics of pure highly concentrated nano-HAP on the demineralized human enamel compared to low concentrated nano-HAP modified by 0.1450% sodium fluoride (NaF).

Materials & Method

Materials Used (Remineralizing Agents):

Types, composition and manufacturer of the remineralizing agents used in this study are shown in Table 1.

Table 1: Types, composition and manufacturer of the materials

Material used	Composition	Manufacturer
20%Nanohydroxy-apetite varnish(ApaCare™).	Synthetic biomimetic nanohydroxyapetite presented in a concentration 20% pure n-HAP. Each varnish vial contains 5 mm pure nanohydroxyapetite.	Cumdente GmbH (Hamburg, Germany).
Remineralizing toothpaste and namely professional ApaCare™ paste.	Synthetic fine nano hydroxyapatite prepared in concentration 3% that was modified by 1450 ppm sodium fluoride (NaF).	

Specimens selection criteria:

Thirty sound permenant premolars with intact buccal surfaces were selected for the study. The teeth were recently extracted for orthodontic purposes.

Specimens preparation& demineralization:

The selected teeth were thoroughly cleaned of debris and washed under tab water then stored in saline until required. The enamel of buccal surface was etched for 30 sec with 37% phosphoric acid gel (Super Etch, SDI Limited, Bayswater, Australia). Then each tooth was rinsed with tab water for 10 seconds and air dried for 5 seconds. Transbond XT primer (3M Unitek, California, USA) was applied to the etched enamel and cured for 10 seconds. Then, Transbond XT light cured composite resin (3M Unitek) was placed on the bracket base. Metal brackets (3MUnitek, California, USA) were bonded to the buccal surfaces of all teeth in the correct position and cured according to manufacturer description. Each tooth was immersed in 10 ml of demineralizing solution for approximately 48 hours.[17,18] This demineralizing solution serves as an acid challenge similar to those generated by acidic bacterial plaque aggregated around orthodontic brackets to induce artificial incipient carious lesions.[19]

- **Demineralizing solution preparation**
  1. The solution composition was based on acetic acid as follow: Acetic acid=4.5083 Calcium=0.4723, Phosphate=0.2722. It was adjusted to the appropriate PH with 50% NaOH after all ingredients were dissolved completely. The PH was set to be 4.4
  2. Selected coded samples from each group were examined by:
- **Environmental Scanning Electrone Microscope Microscope (SEM, Quanta field emission gun FEG 250, FEI Co., Eindhoven, Netherlands) for surface microscopic structure evaluation. Provided by National Research Centre.**
- **Energy Dispersive Analysis of X-rays device (EDAX, FEI Co., Eindhoven, Netherlands).**
  3. Also all coded samples of each group were subjected to the following tests:
- **Tooth color changes assessment by spectrophotometer (Agilent Cary 5000 spectrophotometer) provided by National Institute of standards (NIS)**

Color quantification was done according to the CIE lab system. To compare the color before and after treatment, we incorporated the (**Commision Internatinale de l'Eclairage, L*, a*, b***), **L*** characterizes the lightness and can range from 0(dark) to 100(light). The value of **a*** represents the red(+) green(−) spectrum and **b*** represents the yellow(+) blue(−) spectrum. The **(L*, a*, b*)** values were obtained, and the data were analyzed regarding their lightness and chromaticity values and the corresponding deltas(**ΔL*, Δa*, and Δb***)[20].The total color change(**ΔE**) between two intervals was calculated according to the following equation:

$$\Delta E=[(\Delta L)^2 + (\Delta a)^2+(\Delta b)^2]^{1/2}$$ [21]

The data were tabulated and coded.

- **Surface roughness assessment by Form talysurf i60 specification stylus profilometer provided byNational Institute of standards (NIS)**

In the present study, enamel surface roughness was evaluated at three different points at least around each orthodontic bracket and the mean value of these measurements was used for the statistical analysis. The roughness average parameter (Ra) illustrates the overall surface roughness. An overall decreased(Ra), generally indicates a smooth remineralized enamel surface.[22]

Specimens classification& remineralization:

Each sample was immersed in 10ml of artificially prepared saliva solution [23]that was prepared by mixing the following chemicals in one liter of distilled water:

1) Sodium azide: 0.75 g
2) Potassium mono hydrogen phosphate: 0.804 g
3) Calcium chloride: 0.166 g
4) Magnesium chloride: 0.059 g
5) Sodium chloride: 1.02 g

The decalcified samples were randomly classified into three equal groups: A, B and C according the remineralizing agent used. Each group consists of equal ten coded teeth.

Group A: control group: consisted of ten specimens coded by numbers from one to ten that were immersed for a period of 10 days in artificial saliva and it didn't receive any treatment.

Group B: consisted of ten teeth coded by numbers from one to ten and immersed in artificial saliva for a period of 10 days. During this period, the teeth in this group were treated with synthetic 20% pure nano-HA in the form of varnish namely **ApaCare™ varnish**, which was applied around the peripheral margins of the brackets by varnish brush. It was applied according to manufacturer instructions for about 20 seconds.

Group C: consisted of ten teeth coded by numbers from one to ten, immersed in artificial saliva for a period of 10 days and treated with **medical remineralizing toothpaste ApaCare™**, which consisted of 3%nano-HAP modified by 1450ppm sodium fluoride (NaF) and applied around the bracket margins by soft brush for 3 minutes twice per day according to manufacturer instructions. Between each time of paste application, each tooth was rinsed with distilled water and was cleaned by soft dental brush around the bracket and the saliva was refreshed daily.

After completion of treatment, the three groups were re-examined with the same previous tests to evaluate the efficacy of nano-HAP in repairing the demineralized enamel layer. The results obtained were tabulated and coded to be statistically analyzed.

Statistical Analysis

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The used tests were

- The Kolmogorov-Smirnov test was used to verify the normality of distribution.
- F-test (ANOVA),for normally quantitative variables, to compare between more than two groups, and Post Hoc test (LSD) for pairwise comparisons.

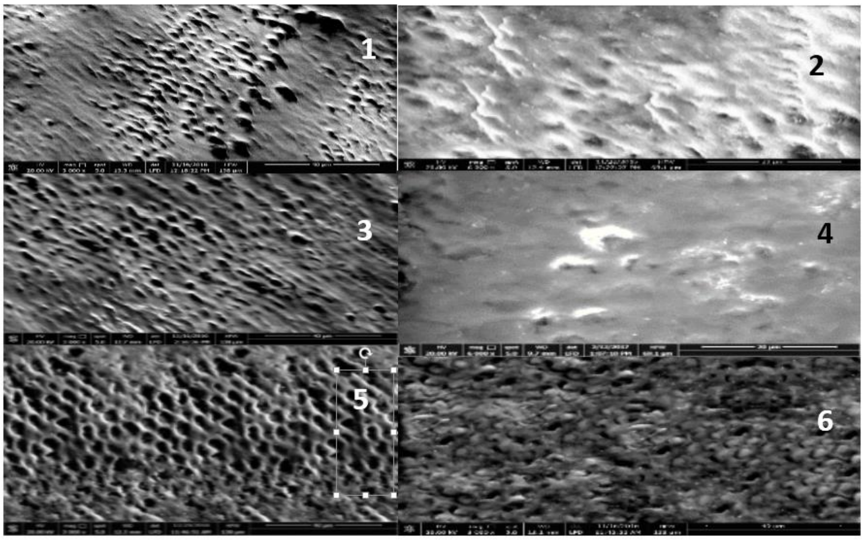
- Paired t-test, for normally quantitative variables, to compare between two periods.
- Kruskal Wallis test for abnormally quantitative variables, to compare between more than two studied groups. Significance of the obtained results was set at 0.05.

Results

Scanning electron microscope analysis (SEM):

After samples demineralization, highly porous enamel surfaces were detected in SEM images (1, 3 and 5) for group (B, C and A) respectively. After treatment by nano-HAP remineralization signs were obviously detected, and decrease in enamel pore size occurred. The porous enamel surface showed a degree of smoothness in both treated groups Band C compared to the control group A as shown in SEM images (2,4 and 6) respectively. (Fig. 1).

Fig. 1. Scanning electron micrograph for randomly selected samples showing ultra-surface morphology of the enamel surface for the 20%nano-HAP treated group B in photos (1&2),3% nano-HAP treated group C (3&4) &for control group A (5&6). Photos (1, 3 and 5) after demineralization, while (2, 4 and 6) after remineralization



The Energy Dispersive X-Ray Analysis Device (EDAX) Analysis:

There was statistically significant increase in Ca% content(p= 0.002) &in phosphorous content(p<0.001) in both nano-HA treated groups Band C compared to the control group A. Significant increase in CaK% was observed in group B in comparison to group C (P=0.036). Also,there were significant increase in phosphorous content (p value =0.003) observed in group B varnish compared to group C as shown in Table 2.

Table 2: Comparison between the three studied groups according to EDAX CaK%, PK%

		Group A	Group B	Group C	P
Before treatment	CaK %	24.79 ± 2.67	28.13 ± 7.04	25.01 ± 2.31	0.453
	PK %	12.06 ± 1.33	12.45 ± 0.46	12.61 ± 0.71	0.630
After treatment	CaK %	24.95 ± 2.65	38.3 ± 7.18	31.39 ± 2.35	0.002
	PK %	12.12 ± 1.33	16.9 ± 0.56	14.72 ± 0.58	<0.001*

ANOVA test was used; Significance between groups was done using Post Hoc Test (LSD).

Spectrophotometric analysis for tooth color changes:

The results of color assessment showed that **L*** parameter significantly decreased after remineralization in both groups B&C (P <0.001 and P=0.001, respectively), while there was no significant change in control groupA (P=0.270). No significant change occurred in a* (P=0.099) whereas b* increased significantly (P < 0.001) in both groups. The results demonstrated a significant difference in the average values of ΔL* and Δb* between groups, however no significant difference appeared in Δa* between groups. A significant difference (P<0.001) in terms of total color change (ΔE) occurred after enamel remineralization as shown in Table 3.

Table 3: Comparison between the three studied groups according to differences in L*, a*, b *& E*

Differences in a*,b*,L*,E*	**Δa***	**Δb***	**ΔL***	**ΔE***
Group A	-0.01 ± 0.80	0.79 ± 0.57	0.0 ± 0.05	0.79 ± 0.57
Group B	0.18 ± 0.22	1.75 ± 0.75	-4.67 ± 0.95	5.04 ± 1.0
Group C	0.09 ± 0.36	1.46 ± 0.79	-1.49 – 4.0	4.25 ± 1.24
P	0.736	0.035*	<0.001***	<0.001***

Kruskal Wallis test was used; Significance between groups was done using Post Hoc Test (Dunn's multiple comparisons test).

Surface roughness analysis:

Surface roughness average (Ra) was revealed that there was statistically significant difference (P<0.001) in the treated groups (Band C) compared to the control group. The highest mean value for average roughness (Ra) after remineralization was for the control group A, while the lowest was for varnish group B as shown in Table 4.

Table 4: Comparison between the three studied groups according to surface roughness (NIS)

Surface Roughness	**Before Treatment**	**After Treatment**	**P**	**% of Improvement**
Group A	0.70 ± 0.06	0.69 ± 0.06	0.039*	-1.21 ± 1.71
Group B	0.69 ± 0.06	0.52 ± 0.05	<0.001**	-24.66 ± 3.04
Group C	0.66 ± 0.04	0.58 ± 0.04	<0.001**	-13.12 ± 1.91

Paired t-test for comparing between before and after Treatment in each group

*: Statistically significant at p ≤ 0.05

Discussion

Natural remineralization of the defected enamel surface through saliva involves mineral gain only in the superficial layer of white spot lesions (WSLs) but it has little repairing effect on the deeper layers of the lesions. It was necessary to potentiate the remineralization process by utilization of synthetic remineralizing agents that supply the tooth with the lost minerals to repair the deeper parts of (WSLs). Recently, biomimetic nano hydroxyapatite has been considered as an alternative to fluoride for re-calcification and repairing of the enamel layer. Its function is to protect the teeth by creation of a new layer of synthetic enamel around the tooth, rather than hardening the existing layer like with fluoride.(13)

SEM, EDAX Assessments:

As detected by SEM& EDAX analysis, nano-HAP has significantly increased the Ca^{+2} and phosphorus levels that penetratedthe micro-pores on the demineralized enamel surface and acted like a scaffold. The nano-HAP particles could attract more calcium and phosphate ions from the encircling remineralizing solution and lodge them into the superficial enamel layer. By doing so, they could fill the gaps in between the enamel calcium crystals, yielding a uniform crystalline enamel structure with high mineral content.

In this current study we have found that pure 20% nano-HAP varnish and 3%nano-HAP modified with 0.145%NaF paste could help in repairing the demineralized enamel surface around the orthodontic brackets. This may be explained by the ability of nano-HAP particles to effectively cover the demineralized enamel surfaces.

Our results have showed that as the concentration of n-HAP increased, the rate and amount of nano-HAP precipitation would also increased, combined with deposition of extensive amounts of $Ca^{2+}$and PO_4^{-3} significantly promoting the remineralization effect. However we have found that when 3%nano-HAP a combined with 1450 ppm sodium fluoride (NaF) it has a significant remineralizing effect. This could be explained by NaF had a synergistic effect on n-HAP in remineralization process as there was a detected & significant changes in calcium and phosphorous levels ($P<0.001$) when comparing group C with control group A.

Tooth color changes assessment by spectrophotometer:

Limited information was available regarding tooth color changes associated with orthodontic treatment with fixed appliances. Our study investigated the color improvement of WSLs associated with orthodontic brackets following nano-HAP treatment in different concentrations. Based on our results, nano-HAP performed better than the control group in improving the esthetic appearance of WSLs.

Our study showed that enamel color variables were significantly affected after nano-hydroxyapetite application, since the ΔL^*&Δa^* values were decreased whereas the Δb^*values were increased representing darker and more yellowish color for the teeth. The mean values of total color change (ΔE^*) were greater than 3.7 units in both groups. This means that the treated enamel with nano-HA has becomes more similar to the sound enamel color.

The nano-HAP could allow crystals repair in the subsurface lesion &decreased the scattering effect of the defected enamel, which affects the perception of the overall tooth color.

In this in vitro study, the color of the artificial white spot lesions (WSLs) were perceived as an area of highlighted white with chalky appearance. Our results showed that enamel remineralization using n-HAP caused observable changes in its color to be similar to the color of sound enamel.

Surface roughness assessment:

Our study showed that the average roughness was decreased significantly ($P<0.001$) when comparing nano-HAP treated groups with the negative control group. This means the nano-HAP has efficiently remineralized the defected enamel surface and restored the enamel surface smoothness to the nearby sound enamel texture. It could be hypothesized that for the chemically induced roughness, nano-HAP with higher concentrations would be more effective.

Conclusion

It could be concluded that poor oral hygiene and diet may, in conjunction with fixed orthodontics, affect the enamel adversely. The mission of an orthodontist is to minimize the risks of the patient having decalcification as a consequence of orthodontic treatment by instructing and motivating the patients for perfect oral hygiene practice. Remineralization potentiality of newly developed synthetic nano-hydroxyapetite on artificially demineralized enamel samples around orthodontic brckets were evaluated

Within the limitations and findings of the present study we can conclude that:

- Acidic demineralization has been adversely affect the enamel surfaces, there obviously detected enamel micro-pores due to subsurface Calcium & Phosphorus minerals loss.
- After demineralization the natural enamel color has lost its natural luster and become dull opaque & chalky white surface.
- The natural saliva was not able to effectively repair the demineralized enamel layer or restore the lost tooth minerals.
- According to the results obtained from the present study we found that the calcium and phosphorus levels has been significantly elevated after remineralization process, however the pure 20%n-HAP has the higher percentage of improvement.
- It was found that nano-hydroxyapetite has also significantly decreased the enamel surface roughness caused by artificial demineralization process & enhanced surface smoothness in both nHAP treated groups, they were relatively similar; however, average roughness was slightly lower in case of higher concentration n-HAP treated group.
- Results of the present study have shown that n-HAP has significantly improved the unfavorable highlighted chalky enamel color resulted after demineralization and could blend it with surrounding sound enamel.

Acknowledgments

The authors express their gratitude to Cumdente company, GmbH for providing the materials used in this research.

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