Research article

Assessment of surface microhardness of enamel surface treated with Nano- HAP serum before and after bleaching

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Abstract: Background: Although bleaching is typically considered a safe procedure, various investigations have found minor negative effects and changes in mineral composition. The aim was to Evaluate and compare the efficacy of using nanohydroxyapatite (n-HAP) serum on surface microhardness of enamel surface before and after bleaching with chemically cured Boost bleaching. Material and methods: Ten sound human permanent upper and lower premolar teeth were used and their roots were removed 2 mm apically to the cementoenamel junction, the crowns were sectioned mesiodistally into two halves buccal and lingual/palatal, the buccal surface was further subdivided into two halves. The samples were embeded in an acrylic resin, resulting in 30 specimens divided into 3 groups: Control group: using Boost bleaching and stored in artificial saliva for 14 days, prevention group: n-HAP serum applied 2-3 min once daily for 10 days followed by bleaching then stored for 14 days in artificial saliva and treatment group: in which bleaching used before n-HAP serum and stored in artificial saliva for 14 days. The samples were subjected to a Vickers microhardness test measured at 4 times: baseline, after n-HAP application, after one day of bleaching and after storage in artificial saliva for 14 days in all groups. The data were analyzed statistically using repeated analysis of variance (ANOVA) test followed by Tukey's test. Results: there was a significant increase in microhardness in the prevention group ($p<0.05$) while there was no significant difference in microhardness readings in control and treatment groups ($p> 0.05$). Conclusion: n-HAP may enhance the microhardness of a bleached enamel surface when used as a preventive and treatment measure. Suggested that a higher increase in enamel microhardness occurs when n-HAP is used for 2-3 min once daily for 10 days before bleaching and maintaining this increase even after storage for 14 days in artificial saliva.

Keywords: remineralization of enamel, nanotechnology, Nanohydroxyapatite, microhardness, bleaching.

Introduction

In recent years, teeth whitening has quickly gained popularity and it is one of the most common dental treatment procedures. Although that bleaching is generally regarded as safe, numerous investigations have discovered minimal adverse effects and changes in mineral composition [1, 2]. Dental bleaching techniques are divided into two types at-home bleaching and bleaching at the office. In-office bleaching procedures are popular for both patients and dentists because they do not need the removal of hard tissues and the aesthetic results can be visible after just one clinical appointment. Additionally, the process is quicker compared to at-home bleaching, thanks to the utilization of a higher concentration of hydrogen peroxide (30%-35%) rather than 10% carbamide peroxide. This higher concentration reduces the application time and ensures effectiveness, making it more user-friendly. [3]. Based on their activation methods, bleaching agents can be categorized into two types: chemically activated bleaching and light activated bleaching. The bleaching process involves the release of reactive oxygen species (ROSs), highly unstable molecules released during bleaching. These ROSs interact with chromophore chemicals present in the tooth structure, breaking them down and thereby lightening the teeth by fragmenting them into smaller molecules[(4)].

This will provide a "whitening effect" by increasing color wavelength absorption and a little amount of color reflection [(5)]. Pain and neurogenic inflammation during teeth-whitening procedures result from the capacity of oxidizing chemicals to trigger nociceptive afferents, which are sensory nerves responsible for detecting harmful stimuli. [(3)]. Other research indicates that bleaching has no clinical relevance for enamel microhardness [(6)]. However, some studies have shown that bleaching affects enamel, alters surface roughness, and reduces microhardness as a result of a non-selective reaction with organic components of tooth tissue [(7-9)]. Another problem is dental sensitivity, which affects more than 70% of patients who have had in-office bleaching previously, as well as increased stains after the procedure because of the roughness generated by bleaching [(10)]. The effectiveness of bleaching, along with its side effects and impact on the enamel surface, is determined by the concentration of the bleaching material and the duration of its usage [(11)]. Various treatment modalities, including fluoride, potassium nitrate, ACP (Amorphous Calcium Phosphate), or nanohydroxyapatite, have been used to manage bleaching concerns [(10)].

The nanotechnology has been developed in dentistry and became used in different branches such as using remineralization of white spot lesions[(12)], arresting and preventing dental caries [(13)], endodontics [(14)], strengthening the restoration [(15)]. Nanomaterials are particles with dimensions on the nanometer scale, their fundamental structural unit occupies three-dimensional space [(16)]. The particle size has been reduced to a range of 0.1 to 100 nm, often accompanied by specific shape alterations. Due to their diminutive size and diameter, phosphate compounds featuring highly bioactive calcium exhibit a greater likelihood of penetrating the porosities of demineralized area [(17, 18)]. Furthermore, no adverse effects on tooth color were observed with n-HAP(nano-hydroxyapatite) treatment, either after in-office bleaching or when combined with the bleaching agent [(19)]. Since hydroxyapatite is the primary inorganic component of teeth, studies on Nano-hydroxyapatite as a biomimetic material for the repair of dental enamel with mineral loss have been investigated and have gained a lot of interest over the years However, there is a lack of additional studies investigating the impact of Nano-hydroxyapatite before and after bleaching procedures utilizing the Boost Opalescence bleaching agent.

The aim of the study is to examine the impact of n-HAP serum on enamel microhardness before and after Boost bleaching. The null hypotheses suggest that n-HAP neither restores microhardness following bleaching nor enhances enamel strength when applied before the bleaching process.

Materials and methods

This study was conducted after acquiring ethical approval No. 497 (in 19-1 -2022). Ten sound permanent human upper and lower first and second premolar teeth were chosen for this study. The selection criteria included the absence of crack, carious lesion , restorations, fluorosis, internal stain, and enamel decalcification [(20)]. For each sample, the teeth were properly cleaned with distilled water before being polished with a non-fluoridated pumice slurry using a rubber cup and a low-speed handpiece to get rid of any residual particles of debris on the tooth surface, then dried with cotton swabs after being rinsed in distilled water. The teeth were kept in a 0.1% thymol solution for one week [(21)]. Then samples were placed in distilled water (DW) until they received the treatment [(16, 18)]. Diamond discs were used to cut away the teeth's roots 2 mm apically from the cementoenamel junction. The crown was then divided into buccal and lingual/palatal halves using a diamond disc under constant water cooling. The buccal surface was further divided into two halves by drawing a line from the cervical line to the tip of the cusp, resulting in a total of 30 specimens [(22)]. The cut specimens were then placed into a custom-made silicone mould (1x1 cm dimensions) and embedded in cold cure acrylic resin (DMP Ltd, Germany) and the natural surface polished. Samples were polished using 1200 grit waterproof silicon carbide abrasive paper for 10 seconds, diamond paste polishing was employed (15 um Diamond Paste) [(23)], and after that, the specimens were tested for surface microhardness. Only enamel samples with comparable baseline Vickers hardness values were chosen for the study to standardize the specimens [24]. The diamond indenter was loaded with a 500 g load and left on the enamel surface for the 30s to calculate the Vickers hardness number (VHN) [19]. G* power software was used to determine the necessary sample size to detect a significant difference between the test treatment, power of study=95%, alpha error of probability=0.05, correlation between time points is 0.5, the effect size of F is 0.40 (large effect size), with 3 groups and time points. The lingual/palatal surface of the teeth served as the control group, while the buccal two halves were separated into prevention and treatment groups (each group contained ten samples).

Nano-hydroxyapatite serum which is a tooth serum PrevDent specially developed to enhance the desensitizing of teeth for a long time and delivers a concentrated dose of nano-hydroxyapatite nHAp.

The groups are as follows:

1- Control group (C): Bleaching was applied on the lingual/palatal surface according to the manufacturer’s instructions.
2- Prevention group (P): Nano hydroxyapatite serum was applied for 2-3 minutes on the buccal surface using the sponge at the end of the tube, then washed off with deionized water. According to the manufacturer's recommendations, this procedure was carried out once per day for ten days. Bleaching was then applied on both the buccal and lingual/palatal surfaces according to the manufacturer’s instructions.
3- Treatment group (T): A bleaching agent was applied on the buccal surface in three cycles, each lasting 20 minutes. After bleaching, the samples were treated with Nano hydroxyapatite serum as per the manufacturer's recommendations.

♦ All samples were stored in artificial saliva for 14 days at 37℃ after measurement 2 (M2)
♦ All experimental groups were subjected to the Vickers microhardness test at four different stages:
M0: measurement was performed before any treatment (at baseline).
M1: After n-HAP serum application for the prevention group, after the bleaching procedure in the treatment group.
M2: After the bleaching procedure in the prevention group and control group, after n-HAP serum application in the treatment group.
M3: After 14 days of storage in artificial saliva.

Statistical analysis

The recorded data was tested by the Shapiro-Wilk test at $p>0.05$ which revealed that surface microhardness is normally distributed among groups. The statistical analysis was performed using Statistical Package for Social Science (SPSS version -22, Chicago, Illinois, USA). Repeated Analysis of Variance (ANOVA) and Tukey Honestly Significant Difference (Tukey's HSD) was used. Level of significance was set at $P<0.05$.

Results

Microhardness assessment among groups and phases

A descriptive and statistical test (Repeated measure ANOVA test) of surface microhardness among groups and phases showed in table 1. Table 1 showed that in the control group: surface microhardness increased between phases from baseline to M2 (bleaching), then decreased with no significant change in M3 (storage in artificial saliva), P= 0.518. In the prevention group: Microhardness increased non- significantly from baseline to M1 (n-HAP) followed by significant increase in M2 (bleaching) then decreased slightly in M3 (artificial saliva for 14 days), P=0.001. In the treatment group the microhardness decreased slightly from base line to M1 (bleaching) followed by an increase in microhardness after the application of n=HAP serum (M2) then decreased in M3 with a non-significant difference, P=0.135. In M1, the surface microhardness was greater in the prevention group (n-HAP) than in the treatment group (bleaching), however, there were no significant difference between the two, P=0.848. Surface microhardness in the M2 and M3 phases was higher in group P (bleaching), followed by group T (Nano), and lowest in the control group. These differences are statistically significant (P=0.001 for M2, P=0.007 for M3).

Table 1: Mean and standard deviation of microhardness Vickers value of different groups and different phases of treatment.

Groups		Baseline (M0)	M1	M2	M3	F	P value
C	Mean	287.738		300.400	276.019	0.752	0.518^
	±SD	27.920		32.768	41.456		
P	Mean	259.629	279.300	356.743	355.738	13.416	0.001*
	±SD	52.254	43.706	13.361	31.445		
T	Mean	285.871	274.790	320.386	296.057	2.338	0.135^
	±SD	35.270	42.631	16.141	51.476		
F		1.091	0.038	11.327	6.471		
P value		0.357^	0.848^	0.001*	0.007*		
Levene test		1.230				P value= 0.057^	

C=control, P= prevention, T= treatment, M1= measurement 1, M2= measurement 2, M3= measurement 3 after storage in artificial saliva for 14 days, p- value: probability value, *= significant difference, ^= non-significant difference.

Multiple pairwise comparisons between phases in each group

Multiple pairwise comparisons of microhardness between phases by groups using Tukey's post hoc test showed in (table 2), there was a significant increase when comparing the group P with control and a significant decrease when comparing group P and group T in both phases (P value in M2, C-P= 0.001, P-T= 0.019) (P value in M3, C-P= 0.006, P-T= 0.042), while other findings were not significant.

Multiple pairwise comparisons among phases in the preventive group (P) using Tukey's test

Table 3 using multiple pairwise comparisons between phases in the prevention group showed that the only non- significant result between base- line (B)- M1(Nano) and M2 (bleaching)-M3 while results between other phases were significantly increased (P value B-M2, B-M3= 0.001) (P value M1-M2= 0.002, M1-M3= 0.003).

Table 2: Multiple Comparisons of Surface Microhardness among groups by phases Using Tukey's test.

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	P value
M2	C	P	-56.343	0.001*
		T	-19.986	0.245^
	P	T	36.357	0.019*
M3	C	P	-79.719	0.006*
		T	-20.038	0.655^
	P	T	59.681	0.042*

C=control, P= prevention, T= treatment, M1= measurement 1, M2= measurement 2, M3= measurement 3 = after storage in artificial saliva for 14 days, p- value: probability value. *= significant difference, ^= non - significant difference.

Table 3: Multiple pairwise comparisons of Surface microhardness in (kg / mm^2) among phases in the preventive group using Tukey's test.

(I) Stages	(J) Stages	Mean Difference (I-J)	P value
Base line	M1	-19.671	1.000^
	M2	-97.114	0.001*
	M3	-96.110	0.001*
M1	M2	-77.443	0.002*
	M3	-76.438	0.003*
M2	M3	1.005	1.000^

Discussion

Teeth whitening has become one of the most popular aesthetic procedures in dental offices due to its properties that provide noninvasive aesthetic results rapidly and without the use of anesthetic or a drill. Despite all of the advantages that are associated with using whitening agents, caution needs to be taken. This is because some reports suggested that there may be some adverse effects, such as increased roughness, mineral loss, and a reduction in the mechanical properties of dental tissue [25, 26]. The negative impact of peroxide bleaching treatments on tooth surfaces could be minimized by using n- HAP material. Furthermore, artificial saliva has a limited capacity for remineralization since it might not fully reverse the demineralization of the tooth surface caused by bleaching chemicals [27]. Surface micro-hardness measurement is considered to be a precise, useful, and effective method for determining how de- and remineralization processes have altered the surface of teeth and only need a small portion of the specimen surface to be tested [28]. This method involved using a diamond indenter at a specific force to press the specimen for a specific time. The Vickers indenter is more practical for measuring tooth hardness than the Knoop indenter; since a square form must always be achieved. In addition, an indentation caused by a non-flat surface or by a difference in the hardness of enamel and dentin may be quickly and easily recognized [29]. The beneficial effect of light activation is reduced when 35 percent hydrogen peroxide is used, because there are already enough radicals produced by the chemical breakdown of hydrogen peroxide to react with the pigments in the dental structure and further increase in radicals will not speed up bleaching [30-32]. Activating energy sources should be used with caution during bleaching treatments because their energy is transformed into heat. Increasing the pulpal temperature by more than 5.5 °C may cause irreversible damage to the pulp. Therefore, Boost opalescence chemical bleaching (40% hydrogen peroxide) had been used.

A fundamental component of human tooth hard tissue, hydroxyapatite compensates for more than 60% of the weight of tooth dentine and is the major mineral component of enamel [33, 34]. Pure hydroxyapatite is insoluble due to its large particle size. However, the Nano-sized crystals have a higher specific surface area and a lower crystallinity, more active components can be released [35]. n-HAP is composed of nanoparticles that are between 50 and 1000 nm in size which is structurally and morphologically similar to the apatite crystals found in enamel. This allows easy penetration to the deep enamel pores [36, 37].

Average enamel hardness values range from 250 to 360 VHN. Teeth beyond this range have been eliminated [38]. There was a slight improvement in VHN in the control group after the application of the bleaching agent, but there was no significant difference from baseline measurements this may be related to the fluoride being added in small concentration to the bleaching agent, which may improve the microhardness of bleached enamel [39].

This result is in agreement with a study done by Kutuk et al. (2018), which used a fluoride and bleaching mixture and reported that the higher microhardness readings were assessed one day after bleaching. Furthermore, enamel microhardness was slightly reduced by Opalescence Boost [19]. Even if it has a high hydrogen peroxide concentration (40%), fluoride (1.1%) may play a role in maintaining tooth enamel microhardness. This fluoride concentration can inhibit a decline in enamel surface microhardness [40].

In the control group, the microhardness value reduced from the baseline after 14 days of storage in artificial saliva, however, none of these results were statistically significant. This can be explained by the fact that the remineralizing agents were able to restore initial microhardness values 24 hours after application and couldn’t continue to perform these effect 14 days later, this may be related to the short contact of the remineralizing agents with the enamel. It should be considered that a single application will not supply enough minerals to maintain microhardness recovery. Additionally, it has been shown that bleaching gel products can stay in the enamel and dentine for up to two weeks. Therefore, more remineralizing agent applications might be needed after 24 hours on bleached enamel to preserve the effects [41, 42]. Moreover, a non-significant reduction in microhardness was seen in the treatment group following bleaching. According to a study by Alkhtib et al., 2013 who used BOOST bleaching, there was an increase in enamel hardness when bleaching was applied for a short period, but there was no statistically significant difference from baseline values. Conversely, when bleaching was applied for long durations, there was a significant decrease in enamel hardness indicating that time of bleaching application is an important factor [43]. This was in agreement with the findings of a research done by Sa et al., in 2013 who applied 40% hydrogen peroxide bleaching in two visits with a 7-days delay between them and stored the samples in artificial saliva, human saliva, and deionized water. The bleaching treatments were performed on the first day and repeated after 7 days, every application take 90 minutes. There was no significant change in microhardness between baseline and final values in all groups [44].

In the preventive group, according to the manufacturer's instructions, Nano serum was applied to sound enamel for 2-3 min per day for 10 days. This resulted in a non-significant increase in microhardness, which may be attributed to the limited solubility of n-HAP at the neutral condition of artificial saliva ph=7. Once bleaching was applied, there was a significant increase in microhardness after 1 day and this increase remained even after storage for 14 days in artificial saliva with no significant difference between measurement periods (1 day and 14 days). The fact that n-HAP solubility increased in acidic conditions could be directly related to the possible mechanism of n-HAP in remineralization. Furthermore, it was found that the n-HAP had a high calcium concentration; an increase in the calcium (Ca^{2+}) concentration would increase the saturation of oral fluids with HA, encouraging apatite mineral deposition and ultimately promoting remineralization. This shows that Nano-HA provides a source of free Ca^{2+}, which is necessary for remineralization [45]. The Nano particles have a stronger affinity on enamel surfaces. The small particle size of n-HAP also improves its surface area for binding and enables it to function as a filler to restore minor defects and depressions in the enamel surface. Researchers are uncertain of how n-HAP biomimetic function works, but some suggest it encourages remineralization by creating a new layer of artificial enamel around the tooth or by adding apatite nanoparticles to enamel defects [46, 47]. Others, however, have suggested that n-HAP functions as a calcium phosphate reservoir, maintaining enamel minerals at a supersaturated state, inhibiting demineralization, and promoting remineralization [48]. Full remineralization is not achievable under neutral conditions, although that n-HAP promotes favorable remineralization of the outer enamel; nevertheless, under acidic conditions, n-HAP can greatly speed up the rate, depth of penetration, and extent of remineralization [45]. When n-HAP was applied to previously bleached enamel in the treatment group, there was a significant increase in microhardness, which was followed by a non-significant decrease after storage for 14 days in artificial saliva. However, the microhardness of the last measurement was significantly higher than the base line.

The higher readings for microhardness in the preventive group revealed that usage of n-HAP before bleaching was associated with a significant increase in microhardness of the enamel, which remained even after storage for 14 days in artificial saliva. Therefore, the null hypothesis was rejected. These results were consistent with a study by Kutuk et al. (2018), which reported that when using a mixture of 38% hydrogen peroxide and n-HAP in different application protocols, microhardness and chemical composition measurements were repeated after 1 and 14 days, they found that the use of n-HAP was capable of increasing enamel microhardness and maintaining microhardness values for 14 days after bleaching [19]. The result of the study disagrees with study done by da Costa Soares et al., 2013 who reported that after teeth were bleached with 35% hydrogen peroxide and treated with the application of a nanohydroxyapatite-based agent (Nano-P), stored in artificial saliva, the enamel morphology and microhardness were assessed again after 24 hours and 14 days of post-bleaching treatments, they found that Nano-P-treated samples had statistically the highest microhardness 24 hours after application. After 14 days of the treatment, the microhardness also did not improve. The short-term contact between the remineralizing agents and the enamel may be the explanation for these results. It should be considered that a single application will not deliver enough minerals to preserve microhardness and enamel integrity 24 hours after application [42].

This in vitro study was unable to fully simulate the intraoral condition, which involved dynamic and complex biological factors like saliva and plaque bacterial biofilm as well as natural remineralization. The study's limitation is that it only investigated surface microhardness and used one bleaching protocol, without taking other factors linked to the composition of the bleaching agents into consideration. Further research must be planned to examine different bleaching protocols with or without the use of n-HAP while still preserving the integrity of the dental hard tissues and different storage intervals in artificial saliva.

Conclusion

Within the limitation of this study, it was found that when n-HAP is used as a preventive and/or treatment, it may increase the microhardness of a bleached enamel surface. It has been suggested that n-HAP uses for 2-3 minutes per day for 10 days before bleaching results in a greater improvement in enamel microhardness, which continues to develop even after 14 days of storage in artificial saliva. The maximum value of average hardness and the repair of mineral content, specifically in the preventive group, indicate that n-HAP has sufficient effects for re-mineralizing.

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

M.H.A. contributed to the conception or design of the work and was responsible for the acquisition of data. R.H.J. and M.M.contributed to the interpretation of results. M.H.A., R.H.J. and M.M. drafted the work. All authors approved the final version of the manuscript and are responsible for all aspects of the work.

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تقييم الصلادة الدقيقة لسطح مينا الاسنان المعالج بمصل النانوهايدروكسيباتيت قبل التبييض وبعدة

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المستخلص

الخلفية: اكتسب تبييض الأسنان شعبية سريعة في السنوات الأخيرة وهو أحد أكثر علاجات الأسنان استخدامًا. على الرغم من حقيقة أن التبييض يعتبر عادةً إجراءً آمنًا، فقد وجدت العديد من التحقيقات آثارًا سلبية بسيطة وتغيرات في التركيب المعدني. الهدف من الدراسة هو تقييم ومقارنة فعالية استخدام مصل النانو هيدروكسيباتيت على الصلادة الدقيقة لسطح المينا قبل وبعد التبييض باستخدام التبييض المعزز كيميائياً. المواد والطرق: تم استخدام عشرة أسنان ضاحك أولية علوية وسفلية دائمة بشرية سليمة وتمت إزالة جذورها بمقدار 2 مم قميًا إلى تقاطع المينا مع السمنت، وتم تقسيم التيجان بشكل متوسط إلى نصفين شدقي ولساني / حنكي ، تم تقسيم السطح الشدقي إلى قسمين نصفين ، تم استخدام قالب سيليكون لوضع العينات في راتينج أكريليك ،الإجمالي 30 عينة مقسمة إلى 3 مجموعات (ن = 10 لكل مجموعة): مجموعة التحكم: استخدام التبييض المعزز وتخزينها في لعاب صناعي لمدة 14 يومًا ، مجموعة الوقاية : المصل يتم وضعه من 2-3 دقائق لمدة 10 أيام متبوعًا بالتبييض ثم تخزينه لمدة 14 يومًا في لعاب صناعي ومجموعة العلاج: حيث يتم استخدام التبييض قبل المصل وتخزينه في لعاب صناعي لمدة 14 يومًا. العينات خضعوا لاختبار فيكرز للصلابة الدقيقة تم قياسه 4 مرات: خط الأساس، بعد المصل، بعد يوم واحد من التبييض وبعد التخزين في لعاب اصطناعي 14 يومًا في كل

المجموعات. تم تحليل البيانات إحصائيًا باستخدام اختبار shapiro-wilk واختبار تحليل التباين أحادي الاتجاه (ANOVA) متبوعًا باختبار Tukey (HSD) تم تعيين مستوى الأهمية عند p <0.05. النتائج: كانت هناك زيادة معنوية في الصلادة الميكروية في مجموعة الوقاية (P <0.05) بينما لم يكن هناك فرق معنوي في قراءات الصلابة الميكروية في مجموعتي التحكم والمعالجة (P> 0.05). الخلاصة: قد يعزز n-HAP الصلابة الدقيقة لسطح المينا المبيض عند استخدامه كإجراء وقائي ومعالج. اقترح أن تحدث زيادة أعلى في صلابة المينا الدقيقة عند استخدام n-HAP لمدة 2-3 دقائق لمدة 10 أيام قبل التبييض ومع الحفاظ على هذه الزيادة حتى بعد التخزين لمدة 14 يومًا في اللعاب الاصطناعي.