Original Research Article

Effect of Nano-Hydroxyapatite Bleaching on Human Enamel Surface

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Abstract

Objectives: This in vitro study was designed to investigate the effect of bleaching with nano-hydroxyapatite material on human enamel in term of surface elemental alterations.

Materials and methods: A total of 240 intact non carious human premolars, extracted for orthodontic reasons, were used. Enamel blocks of uniform dimensions (4 mm×4 mm×3 mm) were prepared from the middle third of buccal halves of each tooth. The dental blocks were individually embedded in self-curing colorless acrylic resin. With the external enamel surface exposed for different applications. The dental blocks were randomly divided into two equal groups (n=120), according to the application of the bleaching agent, i.e. either not subjected to bleaching agent (distilled water as control group) or subjected to hydrogen peroxide bleaching agent. Each group was further subdivided into two equal subgroups of 60 specimens each, according to the application of nano-hydroxyapatite. The groups were designed as follows: Group (DW) distilled water; group (HP) hydrogen peroxide; group (HA+DW) nano-hydroxyapatite with distilled water and group (HA+HP) nano-hydroxyapatite with

hydrogen peroxide. The materials used in this study were, hydrogen peroxide bleaching agent (Opalescence extra boost 38%) used as recommended by the manufacturer's instructions, nano-hydroxyapatite material (nhap), mixture between hydrogen peroxide and nhap, and distilled water used as a control group. Samples were evaluated for elemental composition changes using Scanning Electron Microscope and Energy Dispersive X-Ray Spectroscopy (EDS). The elemental composition changes of the enamel surface were calculated for each sample. The data were statistically analyzed using Kruskal-Wallis test was used to compare between the four groups. Mann-Whitney U test was used for pair-wise comparisons between the groups when Kruskal-Wallis test was significant. Friedman's test was used to study the changes by time within each group. Wilcoxon signed-rank test was used for pair-wise comparisons between the time periods when Friedman's test was significant. **Results:** The results revealed regarding the Calcium weight loss there was a statistically significant difference between the groups as HP group showed the highest Ca wt% loss, HA+DW group, HA+HP group then DW group respectively. Regarding phosphate wt% loss there was a statistically significant difference between the groups, however HA+DW group showed the highest mean value increase, then HA+HP group, HP group, DW group showed no significant differences between them. **Conclusions:** Under the limitations of this study, several conclusions could be detected: Adding nano-hydroxyapatite material to peroxide bleaching agents could decrease its detrimental effect on tooth surface and The remineralizing potential of artificial saliva is limited, as it may not repair the whole tooth surface demineralization caused by bleaching agents.

Key words

Nano-hydroxyapatite, Bleaching, Elemental analysis, Demineralization, Spectroscopy.

Introduction

Tooth discoloration, regardless of its origin, is the most important factor in the esthetics of a smile as discoloration is more rapidly perceived than other esthetic abnormalities [1]. The etiology of dental discoloration is multifactorial.

Today's esthetic dentistry seeks to improve the appearance of patients' smiles with a minimally invasive approach [2]. Tooth bleaching is a well-accepted method of treating discolored tooth since the 19th century [3]. One of the most effective bleaching agents is the hydrogen peroxide (HP), whose application in dentistry was described by Harlan in 1884. HP may be applied directly, or produced as a result of a chemical reaction from carbamide peroxide (CP). It acts as a strong oxidizing agent through the formation of free radicals, reactive oxygen molecules, and HP anions. These reactive molecules interact with chromophore molecules and oxidize the macromolecules and pigment stains [4, 5].

(HP) produces local undesirable effects on tooth structures and oral mucosa. In clinical conditions, some transient adverse effects have been reported on the oral mucosa and the digestive tract if the product is swallowed. Local effects may occur on the oral mucosa and dental tissues during whitening, pulp sensitivity, cervical resorption, release of some components of dental restorative materials, and mainly alteration of the enamel surface.

Enamel may be weakened by the bleaching agent. Numerous researches have evaluated the effects of peroxide-containing products on the chemical and physical properties of tooth enamel. However, researches in this area have alterations of surface morphology [6, 7]; have found calcium loss [8] changes in chemical composition, and decrease in hardness [7, 9] and fracture resistance of enamel [10]. The acidity of (HP) is the main cause of the demineralization effect. To overcome the mineral loss some ingredients such as calcium, fluoride, or

amorphous calcium phosphate are added by the manufacturers into the bleaching gel [11, 12].

Hydroxyapatite materials can be used for this approach, the hydroxyapatite material itself is white and they adhere to surface of tooth without affecting the deeper tooth tissue [13]. This white layer will reflect more whitening effects of (HA) on enamel subjected to (HP) bleaching. However, we are not aware of any reports on the efficacy of (HA) under simulated clinical conditions. Therefore, the aim this study was to assess the elemental analysis of enamel surface after usage of (HA) materials.

Materials and methods

Materials:

I.1 Hydrogen peroxide bleaching agent (HP):

In-office whitening Opalescence Extra boost (Ultradent Products, Inc, South Jordan, UT, USA), chemically activated (no light needed), 38% hydrogen peroxide bleaching gel was used. It contains 1.1% fluoride and 3% potassium nitrate; its PH is 7.0. It is supplied as 1.2 ml Opal Boost /Activator syringe**.**

I.2 Hydroxyapatite material (HA):

I.2.a Synthesis of NanoHAP:

Nano-hydroxyapatite (nanoHA) material, to synthesize hydroxyapatite nanoparticles in a systematic, all-round way and basically realized the controllability of the sizes and appearances of the as-synthesized nanoHAP, the high purity and good crystallinity nanoHAP nano-rods. It was prepared via precipitation method in the form of powder. Aqueous solutions of $CaCl_2 2H_2O$ (0.41 M) and $N_3PO_4.12H_2O$ (0.25 M) were prepared using double distilled water. NaOH was added to control PH. The precipitation reaction occurred immediately under stirring. Then the solution was centrifuged for 99 minutes at 4000 rpm and washed to remove the NaCl by-product. The precipitate was then freeze-dried to produce a fine powder.

I.2 Distilled water-nano-hydroxyapatite mixture (HA+DW):

Hydroxyapatite suspension was prepared by mixing nano-hydroxyapatite powder and distilled water (DW) in ratio of 2 gm powder and 1 ml liquid. The pH was measured using the pH meter (Metrohm 691 pH, Switzerland) to be 7.6.

I.3 Hydrogen peroxide-hydroxyapatite mixture (HA+HP):

Nano HA powder was mixed with 38% hydrogen peroxide gel in the ratio of 2 g powder to 1 ml gel. The pH was measured using pH meter to be 5.4.

I.4 Artificial saliva:

The storage media was the artificial saliva. Each liter of the aqueous solution contains 0.2 $CaCl_2$ gm/l, 0.2 KCl gm/l, 0.05 $MgCl_2 6H_2O$ gm/l, 8.0 NaCl gm/l, 1.0 $NaHCO_3$ gm/l, 0.05 NaH_3PO_4HO gm/l, 1.0 Glucose gm/l. the achieved PH value of the artificial saliva was 7.

Methods:

II.1 Teeth selection:

A total of 240 intact non carious human premolars, extracted for orthodontic reasons, were used in this study. Before any treatment, the teeth were ultrasonically cleaned for 3 minutes using ultrasonic scaler (Cavitron Focused Spray slimLINE 1000 (30k)). Teeth with fractures, cracks, caries or other defects were rejected. Then teeth were stored in 0.5% thymol solution with pH=7 for maximum 28 days.

II.2 Samples preparation:

The crowns were submitted to mesio-distal crosscut sections to separate buccal and lingual fragments using the diamond disc. Enamel blocks of uniform dimensions (4 mm×4 mm×3 mm) were prepared from the middle third of buccal halves of each tooth using low speed saw and water coolant. The enamel blocks dimensions were then checked using a precise caliper (Moore and Wright scheffield, UK). The dental blocks were individually embedded in self-curing colorless acrylic resin (Epoxy-Die, Ivoclar AG, Schaan, Liechtenstein) with the external enamel surface exposed for different applications.

II.3 Grouping of the samples: (Table - 1)

A total number of 240 dental blocks were randomly divided into two equal groups (n=120), according to the application of the bleaching agent, i.e.: either not subjected to bleaching agent (distilled water as control group) or subjected to hydrogen peroxide bleaching agent. Each group was further subdivided into two equal subgroups of 60 specimens each, according to the application of hydroxyapatite, i.e.: with or without hydroxyapatite application as per **Table – 1, 2**. The groups were designed as follows:

- Group (DW): distilled water.
- Group (HP): hydrogen peroxide.
- Group (HA+DW): nano-hydroxyapatite with distilled water.
- Group (HA+HP): nano-hydroxyapatite with hydrogen peroxide.

Table - 1: Variables of the study.

Variables	Symbol	Referred to
Bleaching agent	**B0** **B1**	Without bleaching (distilled water) With bleaching agent
Nano-Hydroxyapatite material (nanoHA)	**H0** **H1**	Without Nano-hydroxyapatite (nanoHA) With Nano-hydroxyapatite (nanoHA)

Table - 2: Interaction of Variables.

HA	Bleaching agent		Total
	B0	**B1**	
H0	H0B0	H0B1	120
H1	H1B0	H1B1	120
Total number of samples	120	120	240

II.4 Treatment of enamel

II.4.a Distilled water group (DW):

The samples were immersed in glass container containing 2 ml distilled water for 15 minutes as a control group.

II.4.b Opalescence Xtra Boast group (HP):

Hydrogen peroxide bleaching agent Opalescence extra boost 38% was used as recommended by the manufacturer's instructions, the content was allowed to come to room temperature before mixing the two chemicals together. A thick layer (about 1mm) of 38 % HP was applied on enamel specimen surface for 15 minutes then washing with distilled water was done for 30 seconds to remove residual treatment solution. Then the next 15 minutes treatment was carried out immediately. The same procedure was repeated 3 more times to have a total application of 60 minutes.

II.4.c Nano-hydroxyapatite –Distilled water group (HA+DW):

It was applied to the enamel surface for 15 minutes using dental brush then washed with distilled water for 30 seconds. The same procedure was repeated 3 more times to have a total of 60 minutes application time.

II.4.d Opalescence Xtra Boast-nano-Hydroxyapatite group (HA+HP):

The mixture was applied to enamel surface for 15 minutes then washed with distilled water for 30 seconds. This procedure was repeated 3 more times to have a total of 60 minutes of application.

II.5 Aging in artificial saliva:

All groups were stored in a glass container containing artificial saliva at 37^{o}C over 4 weeks. It was changed daily.

II.6 Testing procedures:

Samples were assessed at baseline (without any treatment), immediately after material application and 4 weeks storage in artificial saliva to evaluate the effect of in office bleaching

with or without the use of nano-hydroxyapatite on elemental composition changes of the enamel surface. Scanning Electron Microscope and Energy Dispersive X-Ray Spectroscopy (EDS) (SEM JEOL 5410 Japan, EDS OXFORD England), with magnification up to 200K, with EDS analysis was used to examine each subgroup and study the elemental compositional changes at baseline and after different treatments. From this analysis the type of elements that were present in the samples in addition to the percentage of each element can be determined. The obtained results were analyzed, digitalized and automatically plotted. The plotted graphs were interpreted and compared.

II.7. statistical analysis:

Results were recorded, tabulated and statistically analyzed. Ra data showed non-parametric distribution so Kruskal-Wallis test was used to compare between the four groups. This test is the non-parametric alternative to one-way ANOVA. Mann-Whitney U test was used for pair-wise comparisons between the groups when Kruskal-Wallis test is significant. Friedman's test was used to study the changes by time within each group. This test is the non-parametric alternative to repeated measures ANOVA. Wilcoxon signed-rank test was used for pair-wise comparisons between the time periods when Friedman's test was significant.

Results

Characterization of Nano-hydroxyapatite (nHA):

Nano HA were found to be to be an average of 25-50nm in length and 7-15nm in diameter **(Figure - 1).** Scanning electron Microscopy revealed that nHA were needle like crystals with aggregates had found during synthesis as shown in **(Figure - 2).** Polycrystalline ring SEAD pattern with HA interplanner spacing was also revealed with TEM denoting polycrystalline material **(Figure - 3).**

XRD pattern shows diffraction lines characteristics of nHA (**Figure - 4).** The straight baseline and sharp peaks of the diffractogram confirm that product was well crystallized. It showed the spectrum of prepared sample which contains Ca, P, C and O. The corresponding molar ratio of various elements was presented in **(Table - 3).** We can also notice a peak of Na where sodium Hydroxide was used to adjust the PH.

Figure - 1: TEM photograph representing nano-hydroxyapatite crystals size and shape.

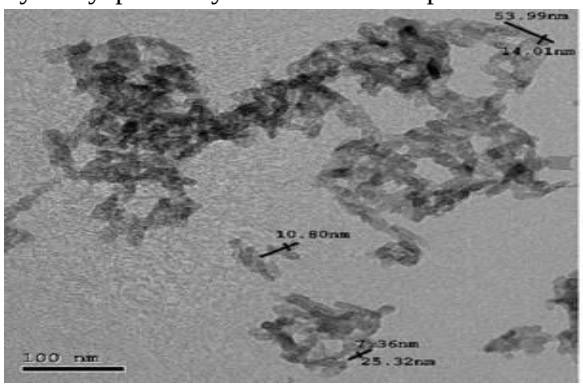


Figure - 2: TEM showing Annealing pattern (fusion of particles).

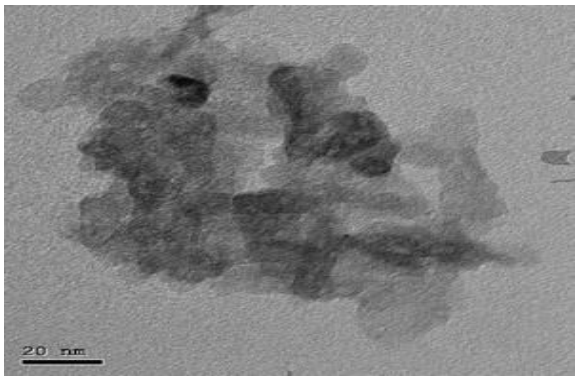


Figure - 3: TEM showing SEAD diffraction pattern.

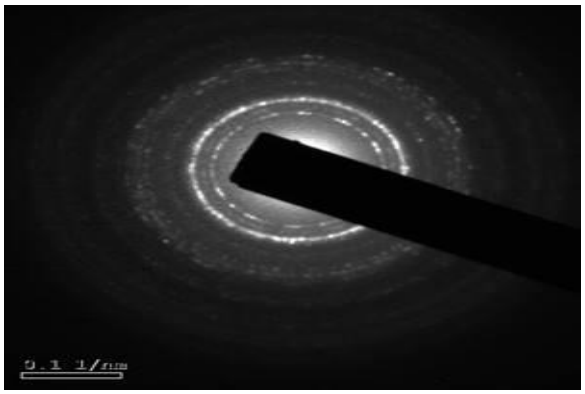
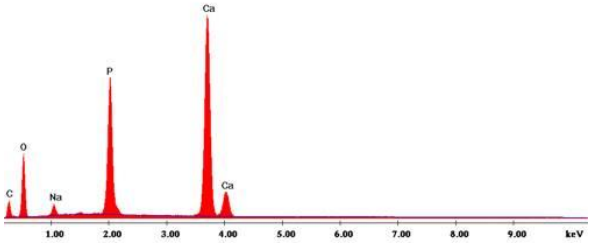


Figure - 4: XRD graph showing elemental composition of Nano-hydroxyapatite (nHAP).



Assessment of elemental composition:

Ca weight %:

Comparison between different treatment modalities:

Results of comparisons between the groups are presented in **Table - 4** and **Figure – 5**.

Baseline values:

One-way ANOVA test showed that there was no statistically significant difference between the groups (*P*-value = 0.115).

Treatment values:

One-way ANOVA test showed that there was a statistically significant difference between the groups (*P*-value = 0.004). Pair-wise comparisons between the groups showed that there was no statistically significant difference between (HP) and (HA+HP) groups; both showed the statistically significantly highest mean Ca weight % values. There was no statistically significant difference between (HA+DW) and (DW) groups; both showed the statistically significantly lowest values.

Storage values:

One-way ANOVA test showed that there was no statistically significant difference between the groups (*P*-value = 0.185).

Comparison between different periods in each tested material:

Results of comparisons between the periods are presented in **Table - 5** and **Figure – 6**.

Table - 3: Elemental analysis of Nano-hydroxyapatite (nHA).

Element	Weight%	Atomic%	K-ratio	Z	A	F
C K	10.14	18.82	0.0224	1.0545	0.2094	1.0007
O K	33.13	46.14	0.0484	1.0367	0.1408	1.0002
NaK	2.16	2.09	0.0075	0.9701	0.3583	1.0018
P K	16.01	11.52	0.1328	0.9586	0.8559	1.0105
Ca K	38.55	21.43	0.3609	0.9655	0.9695	1.0000
Totals	100.00	100.00				

Table - 4: Mean, standard deviation (SD) values and results of One-way ANOVA test for comparison between Ca weight % of the four groups.

Group	(HP)		(HA+DW)		(HA+HP)		(DW)		*P*-value
Period	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Baseline	35.9	2.4	33.5	1.8	34.1	1.7	28.4	3.2	0.115
Treatment	31.3 [a]	1.8	27.9 [b]	1.4	33.2 [a]	3.1	27 [b]	2.1	0.004*
storage	32.2	2.9	30.9	1.4	33.9	1.2	28.2	4.3	0.185

*: Significant at P ≤ 0.05, Different letters in the same row are statistically significantly different according to Tukey's test

Figure - 5: Bar chart representing mean Ca weight % of the four groups.

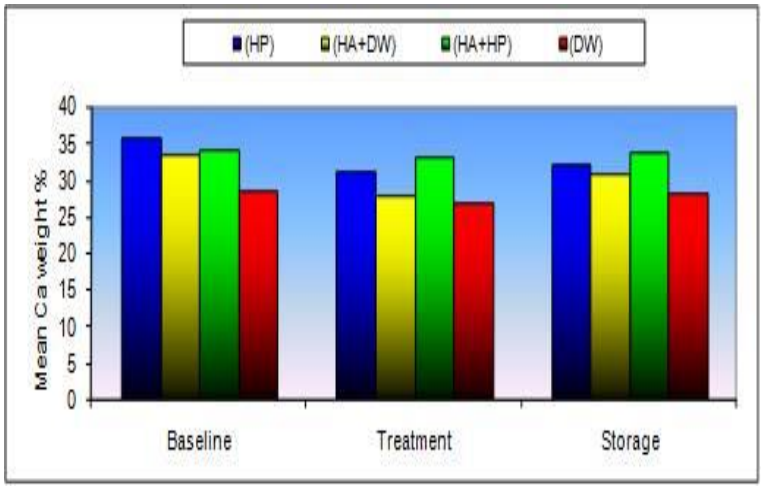


Table - 5: Mean, standard deviation (SD) values and results of Repeated measures ANOVA test for comparison between Ca weight % at the three periods.

Group	(HP)		(HA+DW)		(HA+HP)		(DW)	
Period	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	35.9 [a]	2.4	33.5 [a]	1.8	34.1	1.7	28.4	3.2
Treatment	31.3 [b]	1.8	27.9 [b]	1.4	33.2	3.1	27	2.1
storage	32.2 [b]	2.9	30.9 [a]	1.4	33.9	1.2	28.2	4.3
P-value	0.010*		0.034*		0.538		0.857	

*: Significant at P ≤ 0.05, Different letters in the same column are statistically significantly different according to Tukey's test

Figure - 6: Bar chart representing mean Ca weight % of the three periods.

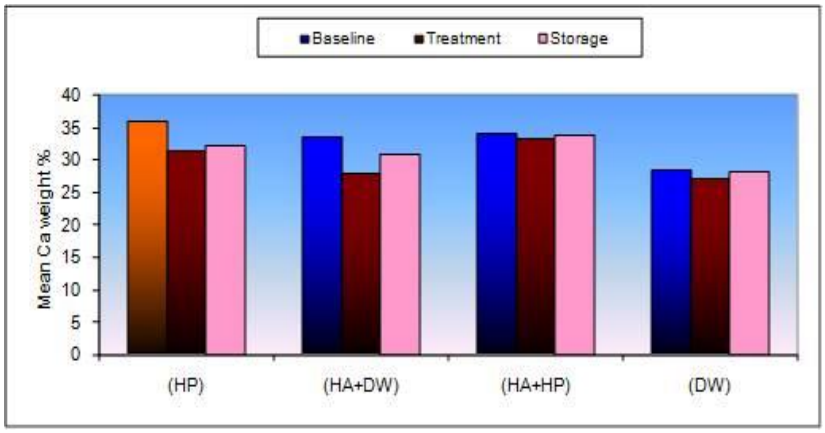


Table - 6: Mean, standard deviation (SD) values and results of One-way ANOVA test for comparison between P weight % of the four groups.

Group	(HP)		(HA+DW)		(HA+HP)		(DW)		*P*-value
Period	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Baseline	13.8	1.1	15.1	0.9	15.7	1.4	16.7	1.2	0.858
Treatment	15.4 [b]	0.8	14.6 [c]	0.8	16.2 [a]	0.4	15 [b]	0.6	0.015*
storage	15.9	1.1	16.2	0.4	16.2	0.1	15.6	1	0.706

*: Significant at P ≤ 0.05, Different letters in the same row are statistically significantly different according to Tukey's test

Figure - 7: Bar chart representing mean P weight % of the four groups.

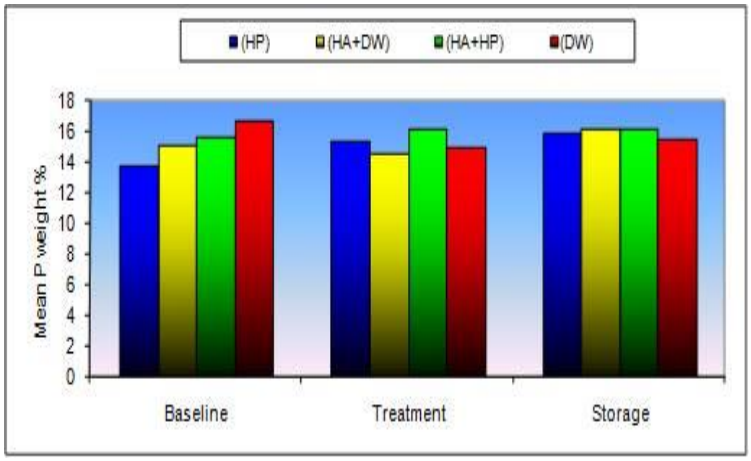


Table - 7: Mean, standard deviation (SD) values and results of Repeated measures ANOVA test for comparison between P weight % at the three periods.

Group	**(HP)**		**(HA+DW)**		**(HA+HP)**		**(DW)**	
Period	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Baseline	13.8	1.1	15.1 [b]	0.9	15.7	1.4	16.7	1.2
Treatment	15.4	0.8	14.6 [b]	0.8	16.2	0.4	15	0.6
storage	15.9	1.1	16.2 [a]	0.4	16.2	0.1	15.6	1
P-value	0.075		0.040*		0.260		0.055	

*: Significant at P ≤ 0.05, Different letters in the same column are statistically significantly different according to Tukey's test

Figure - 8: Bar chart representing mean P weight % of the three periods.

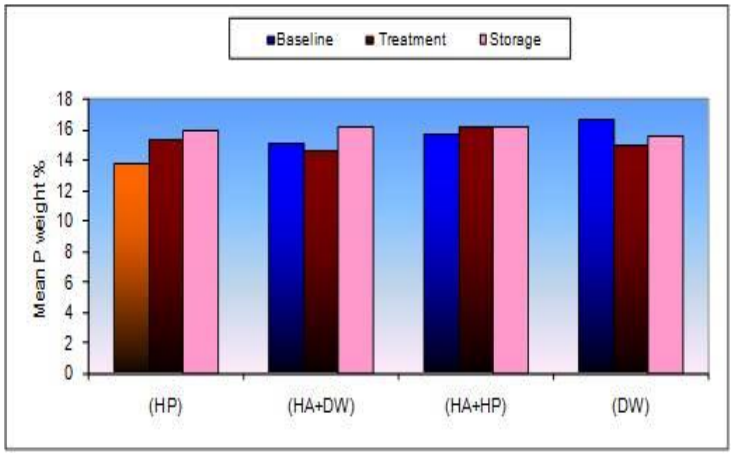


Table - 8: Mean, standard deviation (SD) values and results of One-way ANOVA test for comparison between Ca:P ratio weight % of the four groups.

Group	(HP)		(HA+DW)		(HA+HP)		(DW)		*P*-value
Period	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Baseline	2.6	0.4	2.2	0.6	2.2	0.3	1.7	0.4	0.080
Treatment	2	0.3	1.9	0.3	2	0.4	1.8	0.5	0.375
storage	2	0.2	1.9	0.4	2.1	0.3	1.8	0.1	0.747

*: Significant at P ≤ 0.05

Figure - 9: Bar chart representing mean Ca:P ratio weight % of the four groups.

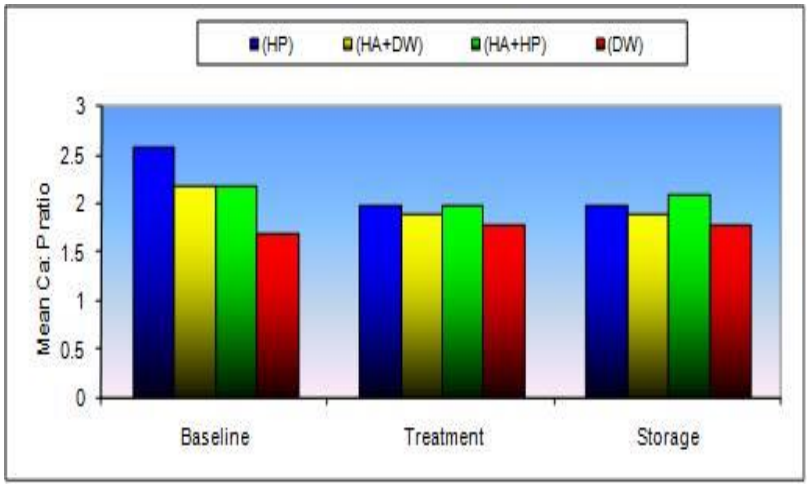
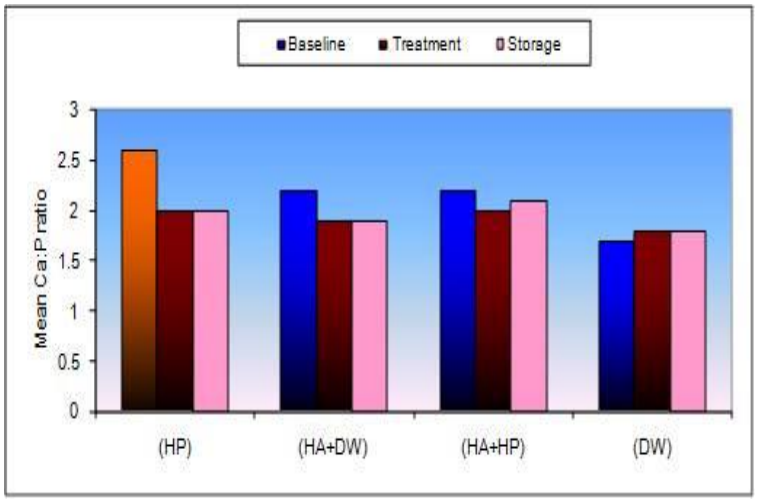


Table - 9: Mean, standard deviation (SD) values and results of Repeated measures ANOVA test for comparison between Ca:P ratio weight % at the three periods.

Group	**(HP)**		**(HA+DW)**		**(HA+HP)**		**(DW)**	
Period	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Baseline	2.6	0.4	2.2	0.6	2.2	0.3	1.7	0.4
Treatment	2	0.3	1.9	0.3	2	0.4	1.8	0.5
storage	2	0.2	1.9	0.4	2.1	0.3	1.8	0.1
P-value	0.481		0.829		0.932		0.950	

*: Significant at $P \leq 0.05$

Figure - 10: Bar chart representing mean Ca:P ratio weight % of the three periods.



(HP) group:

Repeated measures ANOVA test showed that there was a statistically significant difference between the periods (*P*-value = 0.010). Pair-wise comparisons between the periods revealed that there was a statistically significant decrease in Ca weight % Treatment period. There was no statistically significant change in mean Ca weight % in Treatment – storage period. Through the whole study period (storage – Baseline), there was a statistically significant decrease in mean Ca weight %.

(HA+DW) group:

Repeated measures ANOVA test showed that there was a statistically significant difference

between the periods (*P*-value = 0.034). Pair-wise comparisons between the periods revealed that there was a statistically significant decrease in Ca weight % in Treatment period. There was a statistically significant increase in mean Ca weight % in Treatment - storage. Through the whole study period (storage – Baseline), there was no statistically significant change in mean Ca weight %.

(HA+HP) group:
Repeated measures ANOVA test showed that there was no statistically significant difference between the periods (*P*-value = 0.538).
(DW) group:
Repeated measures ANOVA test showed that there was no statistically significant difference between the periods (*P*-value = 0.857).

P weight %:
Comparison between different treatment modalities:
Results of comparisons between the groups are presented in **Table - 6** and **Figure – 7**.
Baseline values:
One-way ANOVA test showed that there was no statistically significant difference between the groups (*P*-value = 0.858).
Treatment values:
One-way ANOVA test showed that there was a statistically significant difference between the groups (*P*-value = 0.015). Pair-wise comparisons between the groups showed (HA+HP) group showed the statistically significantly highest mean P weight %. There was no statistically significant difference between (HP) and (DW) groups; both showed lower mean P weight % values. (HA+DW) group showed the statistically significantly lowest P weight %.
Storage values:
One-way ANOVA test showed that there was no statistically significant difference between the groups (*P*-value = 0.706).

Comparison between different periods in each tested material:
Results of comparisons between the periods are presented in **Table - 7** and **Figure – 8**.
(HP) group:
Repeated measures ANOVA test showed that there was no statistically significant difference between the periods (*P*-value = 0.075).
(HA+DW) group:
Repeated measures ANOVA test showed that there was a statistically significant difference between the periods (*P*-value = 0.034). Pair-wise comparisons between the periods revealed that there was no statistically significant change in P weight % in Treatment period. There was a statistically significant increase in mean P weight % in storage - Treatment. Through the whole study period (storage – Baseline), there was a statistically significant increase in mean P weight %.

(HA+HP) group:
Repeated measures ANOVA test showed that there was no statistically significant difference between the periods (*P*-value = 0.260).
(DW) group:
Repeated measures ANOVA test showed that there was no statistically significant difference between the periods (*P*-value = 0.055).

Ca:P ratio weight %:
Comparison between the groups:
Results of comparisons between the groups are presented in **Table - 8** and **Figure – 9**. One-way ANOVA test showed that there was no statistically significant difference between the groups (*P*-value = 0.080).

Comparison between different periods in each tested material:
Results of comparisons between the periods are presented in **Table - 9** and **Figure – 10**. Repeated measures ANOVA test showed that there was no statistically significant difference between the periods (*P*-value = 0.481).

Discussion

Bleaching has been accepted as one of the most effective methods of treating discolored teeth and is considered to be a conservative approach toward obtaining esthetic or cosmetic results

compared with other methods such as veneering or crowning. Over time tooth bleaching has undergone great development, and during the last decade different bleaching techniques have been used [14].

In this study, the effect of nano-hydroxyapatite with or without bleaching on elemental composition of enamel was evaluated. Furthermore, the effect of aging in artificial saliva for a period of 4 weeks was assessed.

In addition to the bleaching materials that are based on various peroxide compounds, which may have some detrimental effects, scientists have been searching for alternative approach [15]. Therefore, Nano-hydroxyapatite (nanoHA) material was also used, as it is considered one of the most biocompatible and bioactive materials, which has gained wide acceptance in medicine and dentistry recently [16]. Owing to its chemical and structural similarity with natural bone and tooth mineral [12], not having irritation effects, and also eliminating the need to replace or change old restorations as in case of bleaching [15]. Combination of Nano-hydroxyapatite and hydrogen peroxide was used, as it was assumed to reduce enamel demineralization caused by hydrogen peroxide alone. A placebo agent (distilled water), with no expected effect was used as control.
Toward this goal, this in vitro study was designed to test the effect of bleaching with hydroxyapatite material on human enamel in term of elemental alterations, which were absolutely important for the scientific proof of clinical situations.

In order to achieve this goal, an accurately designed protocol of the methodology was adopted. The in vitro investigations can help in the estimation of the in vivo usability of new dental materials and products.

All groups were stored artificial saliva at 37^{o}C during the test period (4 weeks) [14], due to its remineralization potential which exists in saliva substitutes that contain Ca2+ and PO4-3 [14, 17], and to assess any possible rebound effect following discontinuation of treatment. The artificial saliva used in this study was replaced every day to maintain constant ion concentration [14].

In this study, each sample was evaluated using Scanning Electron Microscope and Energy Dispersive X-Ray Spectroscopy (EDS), as it is a popular technique for examining dental tissues or materials [18].

In an attempt to standardize the assessment, the same sample was evaluated before different treatment, after and 4 weeks later. Quantification of the calcium and phosphate group in hydroxyapatite was done, as it is good indicator of the degree of mineralization of enamel [19]. While studies on the effects of bleaching on morphological changes to tooth tissue are contradictory, it is generally agreed that peroxides can alter tooth mineral contents [20].

Regarding Ca wt% loss between different periods in each tested material, in (HP) group, there was a statistically significant difference between the periods; there was a statistically significant decrease in Ca weight % Treatment period. This was attributed to Alterations in the mineral content of enamel and dentin might occur due to the acid properties of these materials and their components [17]. There was a non-statistically significant increase in the mean Ca weight % in Treatment – storage period, which was attributed to Although the artificial saliva used in laboratory studies can present some remineralization capacity39, it is important to note that the dynamics of saliva/enamel interaction is a difficult factor to fully replicate in laboratory research [21].

Through the whole study period (Baseline–storage), there was a statistically significant decrease in mean Ca weight %. This may be attributed to the decreased remineralization effect of the artificial saliva as mentioned before, together with that the fact that if the calcium loss is too high, the surface cannot be mineralized.

These findings are in agreement with Tezel et al., 2007 [14].

On the other hand, this result was in disagreement with Oltu, et al. [22]; Shannon, et al. [23]; that stated that the artificial saliva containing calcium and phosphate had a reminerlization potential. (HA+DW) group, showed a statistically significant difference between the periods. There was a statistically significant decrease in Ca weight % in Treatment period. This may be attributed to the nHAP particles adhered to the enamel surface and formed a protective layer for the underlying enamel [12], so the surface was covered with hydroxyapatite nanoparticles and therefore Ca wt% was decreased as the measurement was actually not representing the actual calcium content of the tooth surface. Unfortunately, no papers were found in the literature investigating or discussing the Ca wt% of enamel surface treated with nHAP.

Regarding Ca/P ratio weight %, the result revealed that there was no statistically significant difference between the periods. This was found in disagreement with Santini, et al. [19]; Lee, et al. [24]; who stated that there was significant decrease in Ca/P ratio, due to that artificial saliva contain phosphate, which had the potential effect of reversing the effect caused by the bleaching gels. However, this introduced a variable. Calcium release into the HP solutions from enamel was more than that of phosphorous at all concentrations which effectively will reduce Ca/P ratio in the bleached samples.

Conclusion

Under the limitations of this study, several conclusions could be detected:

- Adding nano-hydroxyapatite material to peroxide bleaching agents could decrease its detrimental effect on tooth surface.
- The remineralizing potential of artificial saliva is limited, as it may not repair the whole tooth surface demineralization caused by bleaching agents.

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