Original Article

Comparative Study of Diode Laser Enhanced Remineralization by Fluoride and Nanohydroxyapatite of the Demineralized Human Enamel

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ABSTRACT

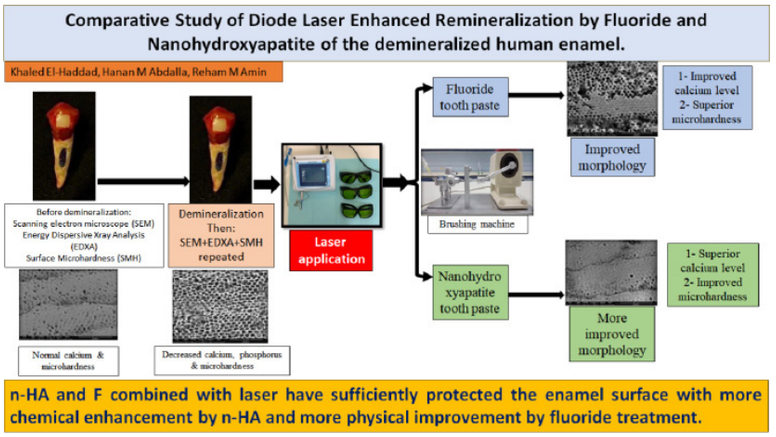
Introduction: Remineralization of the initial enamel lesions is an important preventive procedure that applies several agents and techniques. Many remineralizing agents and techniques have been reported, and there is controversy about the optimum procedures. Fluoride is considered a gold standard in remineralization, but its limitations necessitated the innovation of new agents.

Aim of the Work: The present study aims to compare the effect fluoride versus nanohydroxyapatite on enamel remineralization enhanced by a diode laser.

Materials and Methods: Eight enamel samples of human premolars were subjected to hydrochloric acid demineralization, followed by laser application. The samples were equally divided into two subgroups, one subjected to fluoride and the other subjected to nanohydroxy apatite toothpaste. The assessment was done by scanning electron microscope, energy dispersive X-ray Analysis, and Micro Vickers Hardness Tester before and after demineralization; and also after remineralization. The outcomes of samples in each stage were considered as a separate group.

Results: Demineralizing solution caused observable defective enamel surface and significantly decreased microhardness and calcium weight percentage. The remineralization by nanohydroxyapatite revealed a smooth enamel surface with minimal defects by scanning electron microscope more than fluoride, which showed a remineralized enamel surface alternating with areas of erosive defects. nanohydroxyapatite caused enhanced calcium repair by energy dispersive X-ray Analysis more than fluoride. Buccal enamel surface microhardness was more improved in the fluoride-treated samples than in those treated with nanohydroxyapatite. The results were statistically significant in the calcium wt % changes and the microhardness (*p-value* <0.05).

Conclusions: Each of nanohydroxyapatite and fluoride sufficiently protected the enamel surface. The chemical composition of enamel was more improved by nanohydroxyapatite. While the microhardness was more enhanced by fluoride.



Graphical Abstract

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Key Words: Dental enamel, diode laser, hydroxyapatite, tooth remineralization, topical fluorides.

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INTRODUCTION

The dental enamel is the hardest human tissue which does not remodel or regenerate after being damaged[1]. Acid invasion of enamel caused by many factors, such as soft drinks, increases the occurrence of irreversible enamel defects, which causes aesthetic and functional complications[2,3]. As a result, reducing the likelihood of acid erosion along with other physical changes to enamel required making it more acid resistant[4].

Topical fluoride application is a gold-standard remineralizing agent that performs marked early remineralization during the first ten days after application[3,5]. Several fluoride formulas revealed effective inhibition of enamel demineralization caused by carbonated drinks[6]. Using fluoridated toothpastes has been proven to increase the remineralization of the enamel subsurface defects[7]. Furthermore, a combination of fluoride and other agents, such as casein phosphopeptide-amorphous calcium phosphate, prevented the demineralization effects on the enamel surface[8].

However, some limitations were reported on fluoride, including its effect limited to the enamel layers near the surface, the decreased effectiveness in pit and fissure caries, and the need for low pH and lack of biofilm in erosive lesions for effective action of fluoride. Additionally, there is a potential for adverse effects of overexposure, such as fluorosis[9-11]. These limitations generated many studies examining the efficacy of remineralization alternatives to fluoride[12].

One of the fluoride alternatives is nanohydroxyapatite (nHA), which has excellent nontoxicity and biocompatibility of hydroxyapatite (HA), constituting about 95% of enamel structure by weight. Moreover, the biosorption properties of HA can be modified by adjusting its degree of crystallinity, which is accomplished by implementing advanced synthesis with a nano-size crystal control[13]. Nanohydroxyapatite caused an increase in the microhardness and improvement in calcium and phosphorus content of the enamel surface, which indicated the remineralizing capability of nHA[14].

Laser, which refers to Light-Amplification by Stimulated Emission of Radiation, is involved in dental procedures. A diode laser has been reported to enhance the enamel criteria, increase its microhardness, and preserve its structural integrity[15,16]. There is a positive concomitant action between fluoride and laser in enamel integrity maintenance and caries prevention[17]. A provisional study was performed regarding the enhancement of microhardness of the demineralized enamel by fluoride toothpaste after diode laser[18]. Laser therapy, along with non-fluoride remineralizing agents like nanohydroxyapatite, showed higher remineralizing effects on the carious initial lesions, increasing the acid resistance of the enamel[19]. To our knowledge, there is a need for more qualitative and quantitative studies to compare the fluoride and non-fluoride remineralizing agents in conjugation with laser. Our research set out for comparison the diode laser-enhanced remineralization potential based on the results obtained by using topical fluoride and nanohydroxyapatite on demineralized human enamel.

MATERIALS AND METHODS

Samples

The Research Bio-Ethical Committee of the Faculty of Dentistry-Ain Shams University has approved the research proposal (Approval number: FDASU-Rec –ID061401). Eight enamel regions were used in this study obtained from four human premolars which were free of caries. The premolars were assessed by light stereomicroscope to confirm the absence of defects on the buccal surfaces. Four mm2 window was drawn on the buccal surface middle 3rd and the remaining part of the surface was shielded by acid resistant nail varnish. All the study tests were applied to each premolar on either side of the box (two readings for each premolar); thus, the sample size of each group is n 8 (two readings for each premolar); thus, the sample size of each group is = 8.

All samples were assessed using scanning electron microscope (SEM), energy dispersive X-ray analysis (EDXA), and Micro Vickers Hardness Testing (MVHT). Each sample was used in all phases of the study, and the results of all investigations in each phase were considered as a different group.

Experimental Groups

Group I (Control): The teeth were examined before any treatment using SEM, EDXA, and MVHT.

Group II (Demineralization): The same samples were exposed to a demineralizing agent (hydrochloric acid), and then the three tests were repeated.

Group III (Remineralization): The demineralized samples were exposed to diode laser for thirty seconds with 980 nm wavelength and then split down the middle in two equal sections based on the applied remineralizing agents:

- Subgroup IIIA: samples brushed with Fluoridated toothpaste (containing sodium Fluoride 0.315% w/w) for 140 seconds.
- Subgroup IIIB: samples brushed with n-HA-containing toothpaste for 140 seconds. Then, the tests were repeated on the remineralized samples.

Sample size verification was done by G Power version 3.1.9.7 guided by previous studies[12,20]. by assuming an alpha level (0.05) and beta level (0.2), hence, the power = 80 % and effect size (0.75). The sufficient sample size was eight samples per group.

Demineralization procedure

Hydrochloric acid 1mol/L, 15% concentration, and PH average of 4.5. The acid was statically applied for 5 minutes over the surface then washes with distilled water and pats with absorbent paper to remove excess moisture[21].

Diode laser

A laser device (SIMPLER LASER, made in Italy) was used. As soon as the tooth is secured in the hard wax, the beam was directed at right angle to the tooth surface. A two-centimetre gap was maintained among the laser probe tip as well as the tooth specimen[22]. Samples were stored in distilled water till the subsequent step.

Brushing apparatus

For standardization of brushing technique, a tooth brushing apparatus was used[23]. The apparatus consisted of a double-pane balance, brush holder, weight holding pane, samples holding pane, and a gearbox to decrease the motor speed to two cycles/second with a crankshaft in addition to linking rod attached to a slider to transform the rotation movement to standardized linear 5 mm movement. The samples were held on a balance where the other side contained a weight (Figure 1).

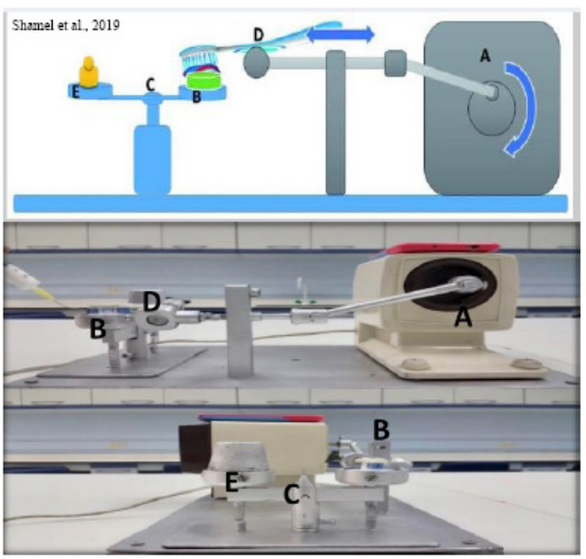

Fig. 1: Brushing device (A) Gearbox (B) samples-holding pane (C) double-pane balance (D) brush holder (E) weight holding pane.

The brushing speed was 120 hits/min with a 250g load. The specimens were brushed with the assigned toothpaste for 140 seconds, simulating two weeks of brushing twice daily for 5 seconds[24]. The toothpaste has been mixed with distilled water (toothpaste to water ratio was 2:1)[25]. After brushing, samples were rinsed in running water for 3 minutes and left to dry in open air.

SEM examination at 20 Kilo volts (KV) using the secondary electron LFD detector under magnifications 1000x, 2000x. SEM model is QUANTA 250 FIELD EMISSION GUN, FIELD ELECTRON AND ION COMPANY, Netherlands. The SEM was attached to the EDXA Unit (Energy Dispersive X-ray Analyses) with an S-UTW detector (EDXA Inc., Mahwah, NJ, USA) model 2007. Surface weight percent of calcium and phosphorus were measured.

The enamel surface microhardness (SMH) was measured as Vickers hardness number (VHN) using an automatic digital microhardness tester (Micro Vickers Hardness Tester, Wilson Hardness model: TUKON1102). Each sample was tested by three indentations utilizing a single load of 200 grams with a holding duration of fifteen seconds. The acquired SMH was calculated as the average value of the three measurements. To prevent measurement mistakes, it is necessary to maintain a minimum gap of 150 µm among each adjacent indentation.

Statistical analysis

Statistics was evaluated by Statistical Package for the Social Sciences (SPSS) version 26. Data was tabulated using mean and standard deviation. Group comparisons were done by analysis of variance (ANOVA) followed by post hoc Tukey test for multiple comparisons. The distinction was considered statistically significant if the *P-values* were below 0.05.

RESULTS

Scanning electron microscopic (SEM) examination of enamel surface topography

The control group, Group I, had an enamel surface that was mostly even and devoid of erosive damage. However, there were noticeable horizontal lines at regular intervals on the surface, known as perikymata (Figure 2a). The enamel surface was smooth with minimal occasional non-pitted dark spots (Figure 2b). SEM examination of demineralization group (Group II) showed scattered porosity in the enamel surface with different forms, including linear depressions (Figure 3a), crater-shaped cavities and fish scale depressions with defects in the exposed enamel rods' cores. Occasional regions of fused affected rods were observed (Figure 3b).

Group III, subgroup IIIA (fluoride) displayed patterns of enamel surface remineralization alternating with areas of erosive defects with linear fissure-like appearance (Figure 4a). Upon closer examination, a distinct honeycomb pattern was observed, indicating the erosion of the prismatic enamel core as well as the protrusion of the interprismatic enamel. Occasionally, other areas showed deep crater-like depressions (Figure 4b). Other regions showed wider remineralization (Figure 4c), and fish scale patterns with calcified enamel rods were detected (Figure 4d).

SEM examination of subgroup IIIB (n-HA) revealed a smooth enamel surface in most regions (Figure 5a). Minimal shallow non-cavitated erosive spots were observed comparable to those in the control group (Figure 5b). The enamel rods on the non-porous enamel surface showed a fish scale appearance with no core defects (Figure 5c). The inter-rod region displayed clear, thick, hypercalcified boundary borders around the core, which showed minimal defects (Figure 5d).

Outcomes of enamel surface mineral content

The statistical analysis of the enamel surface minerals

showed the highest surface calcium weight % in group I, subsequent to group III, while group II had the lowest value. The variance amongst groups/subgroups was statistically significant in the calcium weight %, while the phosphorus weight % and the calcium-phosphorus ratio showed non-significant differences. By comparing every two groups/subgroups in the parameters, the differences were statistically significant between groups II, group I and subgroup IIIB in the calcium weight %. All the remaining comparisons revealed statistically non-significant differences (Tables 1-3, Figure 6-8).

Results of enamel surface microhardness

The examined groups and subgroups were arranged in a decreasing sequence, where nanohydroxyapatite was the highest followed by control then fluoride subgroup while the demineralization group showed the least value. There was a significant distinction seen amongst the groups as well as subgroups (p under 0.05). When comparing each pair of groups or subgroups, there is a significant variance among all groups (p below 0.05), except for two pairs: group one compared to Subgroup IIIA and Subgroup IIIA versus Subgroup IIIB. (Table 4, Figure 9).

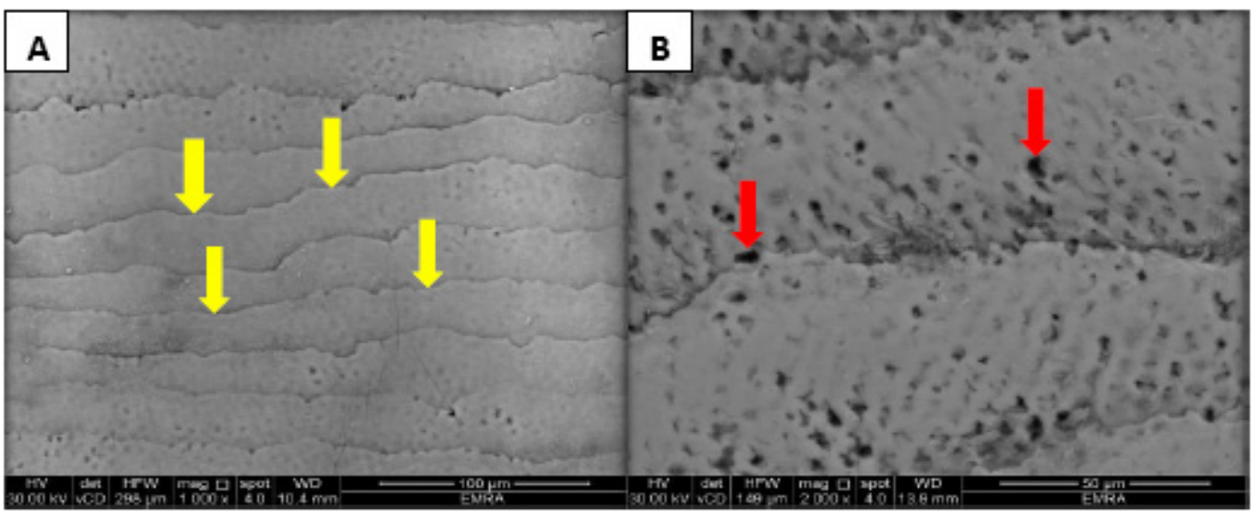

Fig. 2: Scanning electron micrograph of control group showing (A): perikymata (yellow arrows) (B): smooth enamel surface with minimal occasional non-pitted dark spots (A: x1000, B: x2000).

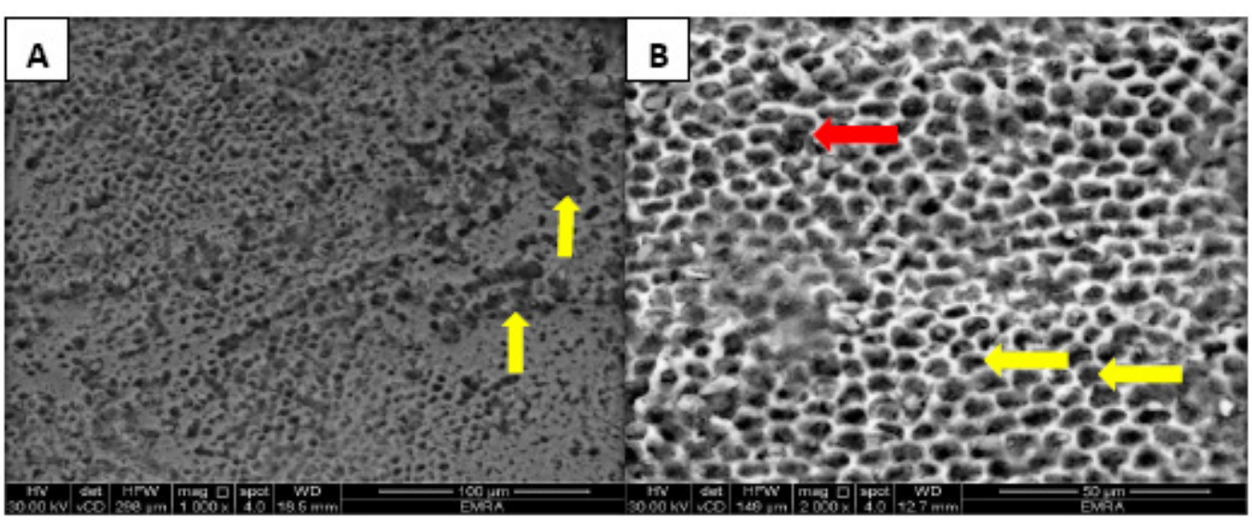

Fig. 3: Scanning electron micrograph of demineralization group showing (A): crater-shaped cavities (yellow arrows) and linear depressions (red arrow). (B): Fish scale depressions with core defects in enamel rods (yellow arrows) and fused rods (red arrow) (A: x1000, B: x2000)

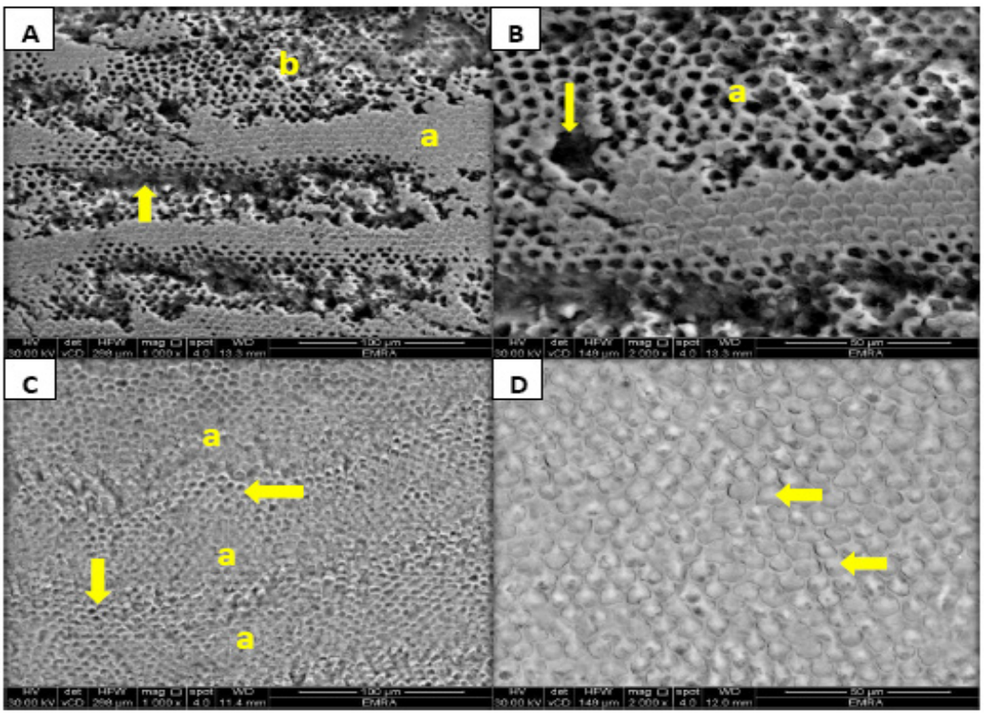

Fig. 4: Scanning electron micrograph of subgroup IIIA showing (A): Remineralized enamel surface (a) alternating with areas of erosive defects (b) with linear fissure-like appearance (arrow). (B): Honeycomb structure (a) with eroded enamel-rod core and projected interprismatic enamel. Deep crater-like depressions (arrow). (C): Wide areas of enamel remineralization (a) with narrow lines of defective enamel rods (Arrows). (D): Fish scale patterns with calcified enamel rods and a few rods with depressed cores were detected (Figure 4c and 4d) (A, C: x1000, B, D: x2000).

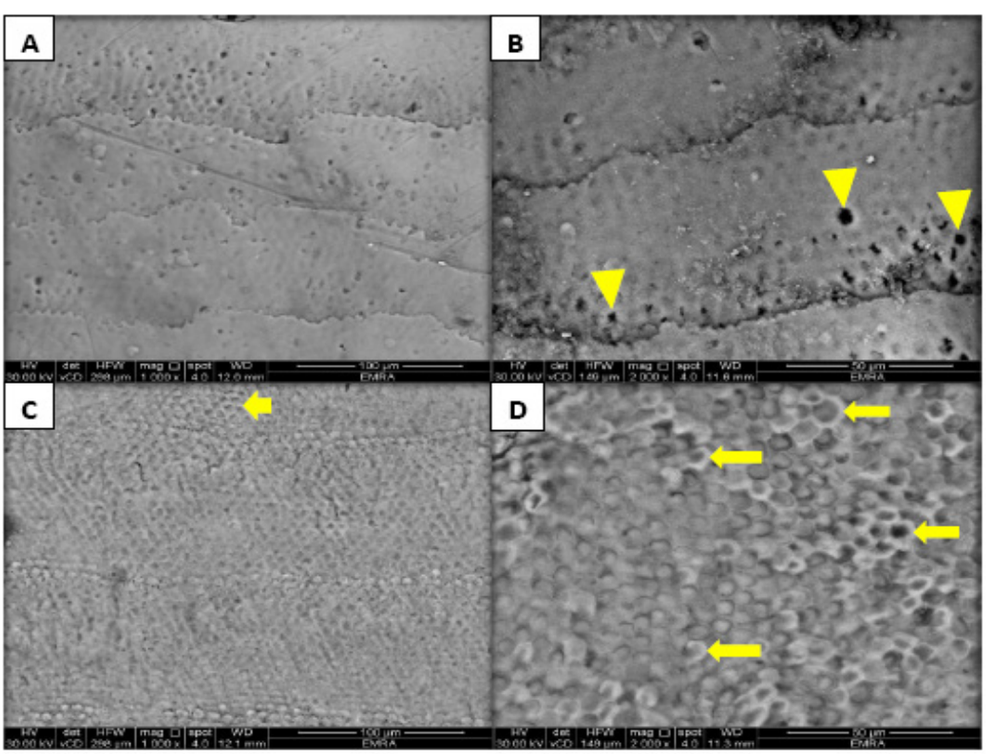

Fig. 5: Scanning electron micrograph of subgroup IIIB showing (A): smooth enamel surface in most of regions. (B): Minimal shallow non-cavitated erosive spots were observed (arrowheads) (C): Non-porous enamel surface with regions of fish scale appearance (arrow). (D): The inter-rod region with thick hyper-calcified boundaries (arrows) (A, C: x1000, B, D: x2000)

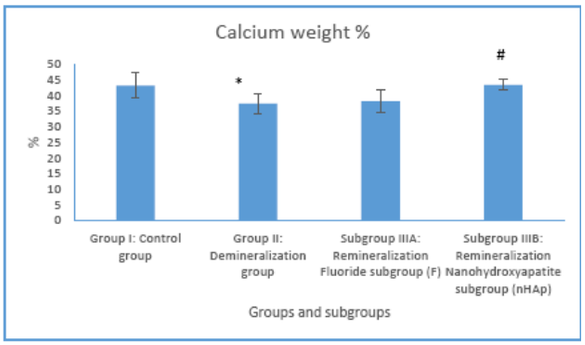

Fig. 6: Bar chart of values of calcium weight % of all groups and subgroups.
*: statistically significant compared to group I;
#: statistically significant compared to group II (P<0.05).

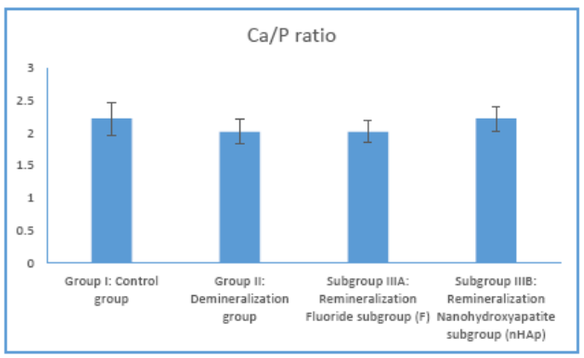

Fig. 8: Bar chart of calcium/phosphorus ratio in all groups and subgroups.

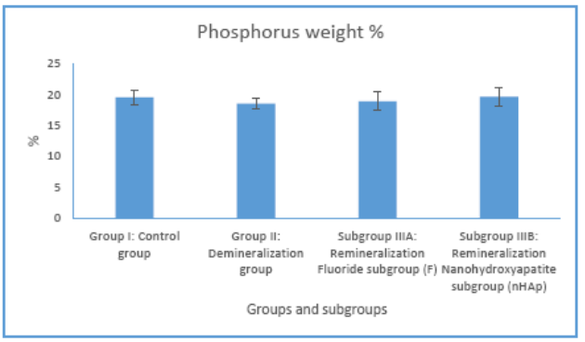

Fig. 7: Bar chart of phosphorus weight % values of all groups and subgroups.

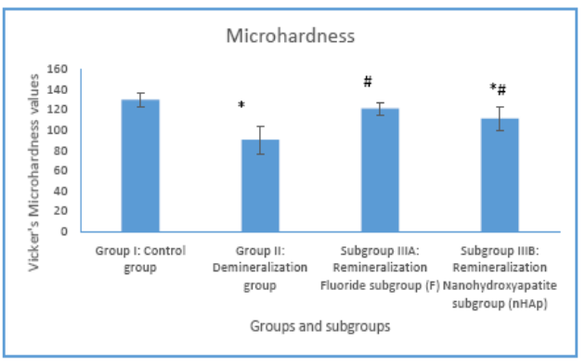

Fig. 9: Bar chart of Vicker's Microhardness values of all groups and subgroups.
*: statistically significant compared to group I (P<0.05).
#: statistically significant compared to group II (P<0.05).

Table 1: Mean and standard deviation of Calcium weight % in all groups and subgroups.

Calcium weight %	Group I: Control group	Group II: Demineralization group	Group III Remineralization group		*P value*
			Subgroup IIIA: Fluoride	Subgroup IIIB: Nanohydroxyapatite	
	43.14±4.04	37.35±3.26	38.13±3.65	43.39±1.63	0.007
Post hoc pairwise comparisons *P value* (between each two groups/subgroups)					
Group I:		0.015*	0.114	0.999	
Group II:			0.982	0.043*	
Subgroup IIIA				0.168	

Values are presented as mean ±SD *, which indicates a significant difference.

Table 2: Mean and standard deviation values of phosphorus weight % in all groups and subgroups.

Phosphorus weight %	Group I: Control group	Group II: Demineralization group	Group III Remineralization group		*P value*
			Subgroup IIIA: Fluoride	Subgroup IIIB: Nanohydroxyapatite	
	19.49±1.16	18.47±0.89	18.86±1.49	19.61±1.43	0.287
Post hoc pairwise comparisons *P value* (between each two groups/subgroups)					
Group I:		0.336	0.818	0.998	
Group II:			0.413	0.413	
Subgroup IIIA				0.804	

Values are presented as mean ±SD * indicates a significant difference

Table 3: Mean and standard deviation values of calcium phosphorus ratio in all groups and subgroups.

Ca/P ratio	Group I: Control group	Group II: Demineralization group	Group III Remineralization group		*P value*
			Subgroup IIIA: Fluoride	Subgroup IIIB: Nanohydroxyapatite	
	2.22±0.25	2.02±0.19	2.02±0.17	2.22±0.19	0.193
Post hoc pairwise comparisons *P value* (between each two groups/subgroups)					
Group I:		0.278	0.448	1.000	
Group II:			1.000	0.440	
Subgroup IIIA				0.560	

Values are presented as mean ±SD * indicates a significant difference

Table 4: Mean and standard deviation values of Vicker's Microhardness values of all groups and subgroups.

Microhardness	Group I: Control group	Group II: Demineralization group	Group III Remineralization group		*P value*
			Subgroup IIIA: Fluoride	Subgroup IIIB: Nanohydroxyapatite	
	129.52±6.38	90.13±13.56	120.85±6.18	111.2±11.59	<0.001
Post hoc pairwise comparisons *P value* (between each two groups/subgroups)					
Group I:		<0.001*	0.523	0.039*	
Group II:			<0.001*	0.015*	
Subgroup IIIA				0.553	

Values are presented as mean ±SD * indicates significant difference

DISCUSSION

The present work focused on enamel remineralization to reverse the initial defects. Although fluoride is a gold-standard remineralizing agent[3,5]; however, it has some reported limitations[9,10]. So, we aimed to compare it with another agent, nano-hydroxyapatite, a promising, nontoxic, biocompatible material with innovative synthesis with nano-size scale[13]. We augmented the remineralizing agents with a portable low-energy (diode laser) because of its low cost, lightweight, and easy handling[22]. We evaluated the enamel changes by various aspects and physical properties using the Micro Vickers Hardness Tester; chemical changes were assessed using EDXA. The surface morphology was investigated by SEM, an appropriate qualitative investigation for the surface topography of initial enamel defects[12].

In the present study, upon demineralizing enamel by HCL, there was a significant reduction in calcium wt % and the microhardness in addition to the clearly observed surface defects in SEM. This is agreed with Lucchese and Gherlone[26], who demonstrated that HCL erodes the surface effectively. The acid attack could be explained by the fact that HCL decreases the PH below the critical value (5.5); hence, the balance between demineralization and remineralization swings to the side of demineralization because of the subtraction of calcium and phosphate ions from the enamel surface. This could lead to erosive defects detected by SEM[27].

Our results revealed improved enamel surface characteristics in the treated group. The Fluoride subgroup showed surface improvements, while some irregular depressions of variable depth and areas were still observed. The nanohydroxyapatite subgroup showed relatively more evident repair. The enamel surface was smooth and regular.

The superior remineralizing potential of nanohydroxyapatite over fluoride was reported by Swarup and Rao[28], who reported that n-HA adhered to demineralization pores. These crystals aggregated and grew into micro-clusters to form an appetite layer covering the enamel rods and inter-rod regions. Furthermore, our findings are consistent with Ebadifar *et al*.[15], who reported that nanohydroxyapatite toothpaste indicated a greater remineralizing effect than sodium fluoride toothpaste[29]. Based on reports, the nano-sized particles bear a resemblance to the apatite crystals found in tooth enamel, both in terms of their shape and crystal structure. Multiple studies have proved that the utilization of nanoparticles effectively inhibits the occurrence of dental caries[15].

Our findings could be explained by a trial done by El-Assal *et al*.[19], which suggested that n-HA could be deposited in the enamel surface defects because of its small size, enabling it to occupy the spaces in enamel crystals.

EDXA examination in our study presented a decrease in surface Ca and P wt% upon demineralization, which was repaired by n-HA application more than fluoride. Our results agreed with Swarup and Rao, who recorded no increase in Ca/P ratio after remineralization with fluoride paste, which could be due to the restricted structural change to the partial substitution of the hydroxyl group by fluoride ions with no change in calcium and phosphate[28].

Regarding enamel microhardness, our study presented a significant rise in the microhardness values in the samples treated with fluoride more than those treated with n-HA. The enhanced surface microhardness with the nanohydroxyapatite and laser was reported by El Assal *et al*.,[19] who stated the combination of laser therapy along with nanohydroxyapatite showed an increase in the enamel microhardness with no alteration of the enamel appearance. This may be attributed to the nanohydroxyapatite ability of tooth surface adherence without affecting or chemically altering the deeper tooth tissues.

Abdulhussein *et al*.,[4] The application of laser followed by fluoride caused more enhancement of enamel microhardness than the application of fluoride alone. Additionally, our provisional study reported the efficiency of fluoride combined laser in the increase of enamel microhardness[18]. Atef *et al*.,[16] reported that there was an increase in enamel microhardness upon the use of either fluoride varnish, n-HA, or diode laser. Many attempts were made to explain the mechanism of action of laser with remineralizing agents. Some authors believe that laser can increase fluoride bonding to the enamel[30]. Others assume that the laser heat causes cracking, which traps minerals and fluoride ions[31]. Further investigations are needed to establish the optimum remineralizing agent, its concentration, application route, and the possibility of combining more than one agent.

CONCLUSION

Our research indicates that either the application of nanohydroxyapatite-containing toothpaste or fluoridated toothpaste, augmented by diode laser, improves the process of enamel remineralization and strengthens its integrity. Nanohydroxyapatite showed more beneficial effects regarding the calcium content than fluoride, while fluoride presented improved microhardness. Evaluating the combination of the two agents is recommended, which could be promising in caries prevention measures.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة مقارنة لإعادة المعدنة المحفزة بليزر دايود بواسطة الفلورايد والنانوهيدروكسي اباتيت على مينا الأسنان البشرية منزوعة المعادن

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المقدمة: تواترت الأبحاث العلمية التي تشير إلى فقد المعادن في طبقة مينا الأسنان بفعل الأحماض. وقد تضافرت الإثباتات العلمية لإمكانية إيقاف التسوس في مهده بواسطة تعزيز إعادة المعدنة عن طريق إضافة عوامل إعادة التمعدن وبالأخص مع استخدام ليزر دايود. وبالرغم أن العلاج بالفلوريد كان يعد حجر الزاوية في استراتيجيات الوقاية من التسوس إلا أنه قد ظهرت العديد من العيوب مما دفع إلى المضيّ في استخدام بدائل كالهيدروكسي أباتيت متناهية الصغر (بحجم النانو) كعامل لإعادة التمعدن. ولكن القليل من الدراسات قد ركزت على فعالية النانو هيدروكسي اباتيت مع ليزر الدايود في منع تآكل الأسنان وبشكل عام في منع هدر الكالسيوم في المينا.

الهدف من البحث: هو المقارنة بين المعجون المحتوي على الفلوريد والمعجون المحتوي على النانو هيدروكسي اباتيت على إعادة التمعدن المدعوم بليزر الدايود على مينا الأسنان منزوعة المعادن.

المواد والطرق: تم انتقاء أفضل ٤ أسنان من الضواحك المخلوعة. تم تعليم مسطح بمساحة ٤ ملم٢ على الثلث الأوسط من السطح الشدقي لكل سنه وتم طلاء باقي السطح بطلاء أظافر مقاوم للأحماض تاركاً المساحة المذكورة مكشوفة لتتعرض لكل خطوات تجربة العلاج بحيث يتم أخذ القياسات مرتين من كل مربع (قياسين من كل سنة) بحيث يكون إجمالي العينات ثمانية حيث يتم فحص الأسنان قبل إزالة التمعدن (مجموعه ١) وبعد إزالة التمعدن (مجموعه ٢) وبعد العلاج بواسطة عوامل إعادة التمعدن بعد تعريض الأسنان لليزر(مجموعه ٣) والتي تنقسم إلى مجموعتين فرعيتين إحداها باستخدام الفلورايد والأخرى باستخدام النانو هيدروكسي أباتيت. تم تقييم الأسنان فيما يتعلق بملامح سطح المينا باستخدام المجهر الإلكتروني الماسح البيئي ومحتوى المعادن في سطح المينا باستخدام محلل الأشعة السينية المشتت للطاقة. أما عن الصلابة الجزئية للمينا فقد تم قياسها باستخدام مختبر الصلابة فيكر.

النتائج: أسفرت نتائج المجهر الإلكتروني للمجموعة مزالة التمعدن تآكل سطح المينا لباب عصيّ المينا. أما مجوعة الفلوريد الفرعية فقد أظهرت أنماطًا لسطح المينا معاد التمعدن مع بقايا لعيوب التآكلية واستمرار تأثر أعمدة المينا. ولكن مجموعة النانو هيدروكي أباتيت الفرعية (٣ب) فقد بدا سطح المينا أملسًا في معظم الأماكن مع وجود بعض البقع التآكلية الدقيقة السطحية ولكن بدون تجويف. ومن خلال تحليل البيانات لسطح المينا تبين زيادة نسبة الكالسيوم في كل من الأسنان المعالجة بالفلورايد و بشكل أكبر في النانو هيدروكسي أباتيت. وقد أظهر قياس الصلابة لسطح المينا تقدم لمجموعة الفلوريد عن النانوهيدروكسي اباتيت.

الخلاصة والاستنتاج: يعزز كل من الفلوريد والنانوهيدروكسي أباتيت التركيب الكيميائي والخصائص الفيزيائية للمينا منزوعة المعادن. مع تفوق معجون النانو هيدروكسي أباتيت على الفلورايد في تأثير الترميم في التركيب الكيميائي غير العضوي. وتفوق الفلورايد في تحسين القساوة الدقيقة للسطح.