Original Article

Impact of Indium Oxide Nanoparticles Mouth Wash in Prevention of Human Dental Enamel Caries (*In Vitro* Study)

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Abstract

Background: Despite the fact that fluoride-based remineralization technologies have dominated preventive dentistry for the past century, new and innovative nanomaterials approaches are transforming the industry. A variety of unique metal nanoparticles (NPs) materials are now be easily available to assist remineralization of the dental enamel surface. **Objectives:** This study was reported to prevent and treat of dental caries using indium oxide NPs suspension solution application in comparison to de-ionized water and sodium fluoride on human tooth enamel by surface microhardness analysis for the surface dental enamel. **Materials and Methods:** Field emission scanning electron microscopy, X-ray diffraction, and ultraviolet (UV)-visible absorption spectrophotometer were used to evaluate the physical characteristics of an indium oxide NPs suspension solution that was created by the laser ablation in liquid. Microhardness Vickers test was used to evaluate the microhardness of teeth. Teeth samples were 60 teeth that were divided randomly into two phases; each phase consisted of prevented and treated groups with indium oxide NPs, control-positive group was treated with sodium fluoride, and control-negative group was treated with deionized water. Each group was consisted of 10 teeth. **Results:** The results showed that there was an increase in microhardness be recorded for both preventive and treatment groups after the application of an indium oxide NPs suspension solution. **Conclusion:** Laser-prepared indium oxide NPs colloidal suspensions were found to be effective as a remineralizing agent to human dental enamel and thus can be considered as an alternative to sodium fluoride mouth wash.

Keywords: Human normal dental enamel, indium oxide nanoparticles, microhardness, sodium fluoride

Introduction

Dental caries is the most common oral health problems among others, which occurs mainly in children and young people. Dental caries is a complex disease process brought on by a microbial imbalance in the oral biofilm in which early detection and attempts are made to reverse or stop dental caries for maintaining tooth structure. Fluoride offers tooth enamel hardness and durability and prevents cavities. It can be used to increase a tooth's resistance to acid attack.

Despite efforts and improvements in caries management, many patients continue to have caries.[1] Nanotechnology can help with dental caries prevention and management by reducing plaque and assisting with initial caries remineralization, because of their distinctive properties, such as their better surface-to-volume ratio and enhanced bioavailability toward cells and tissues.[2,3]

Because of the metal nanomaterials ability to disrupt bacterial metabolism and inhibit biofilm development, in which significant antibacterial action is displayed by metal oxide nanoparticles (NPs) through metal ion release, oxidative stress generation, and non-oxidative processes.[4,5] An important and fascinating transparent metal nanomaterial is indium oxide (In_2O_3), which is used

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in solar cells, photo catalysts, and panel displays. There have been numerous investigations on the production of various structured In_2O_3 materials, including nanotubes, nanowires, nanobelts, and nanofibers, for a variety of uses.[6-8]

Pulsed laser ablation in liquid is one of the most popular dominant technologies to produce pure and stable NPs suspensions due to its low cost, high efficiency, and precision.[9,10] Indium oxide NPs have been created using a variety of methods, including chemical, solvo/hydrothermal, pulsed laser deposition, and others.[11-13] Metal indium oxide NPs have been shown to have antibacterial action. This metal oxide also has therapeutic potential for the treatment of infectious disorders.[14-16]

To date, no foreign study was conducted to investigate the improvement of human dental enamel using laser-prepared indium oxide NPs from that this study was conducted.

Materials and Methods

Preparation of indium oxide nanoparticles suspension by laser ablation

Using 5 mL of pure water in the glass container and a distance of 6 cm from the surface of an indium bulk target, a convex lens with a 5 cm diameter was employed to concentrate the laser beam. The laser pulse's maximum energy was 600 mJ, with such a repetition rate of 1 Hz as well as the duration of about 10 ns. The formed colloidal solution was collected after the NPs were produced using fixed laser energy and the number of pulses with 125 pulses.[11] When the suspension of the produced indium oxide NPs was deposited onto glass slides and allowed to dry, an X-ray was used to characterize them. Cu-K (1.54 eV) was used as the X-ray radiation source at a 2° angle (10°–80°).

The Scherer equation was used to estimate the grain size.[18,19] The expected optical characteristics of NPs were measured using a double-beam UV-visible spectrophotometer at wavelengths between 190 and 1100 nm.[20]

Teeth samples

Maxillary first premolars from Iraqi patients between the ages of 12 and 20 who were seeking orthodontic treatment were used as tooth samples in this study. These teeth were gathered from a variety of Hilla city-based private orthodontic offices. Caries-free teeth were pulled as painfully as possible. Using a rubber cup and hand piece with non-fluoridated pumice and de-ionized water, the teeth were cleaned and polished. To get rid of any pumice that might have been on the surface of the enamel, the teeth were further cleansed with cotton dipped in acetone. Then, the teeth were placed in a plastic screw cup that had been completely filled with deionized water, 0.1% thymol added to prevent bacterial growth.[21]

Study design

The study design was involved (60) teeth which were divided randomly into two phases, each phase with three groups, and each group consisted of 10 teeth as seen in Figure 1.

Preventive phase

- First group: sound teeth without caries-like lesion formation with deionized water (negative control).
- Second group: sound teeth without caries-like lesion formation with NaF (0.05%) mouthwash application (positive control).
- Third group: sound teeth with NPs applications.

Treatment phase

- First group: demineralized teeth with deionized water (negative control).
- Second group: demineralized teeth with NaF 0.05% mouth wash application (positive control).

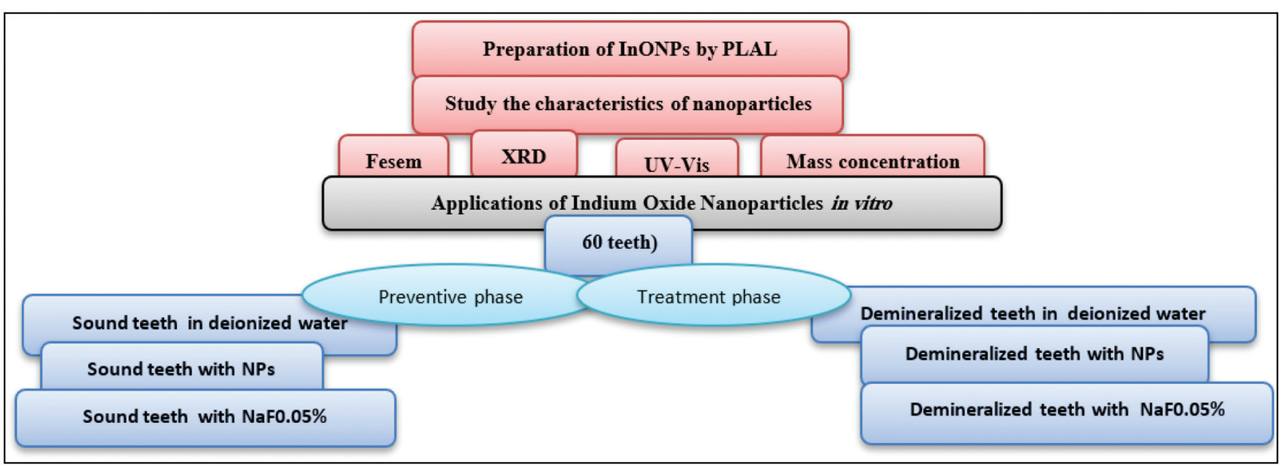

Figure 1: Design of the study

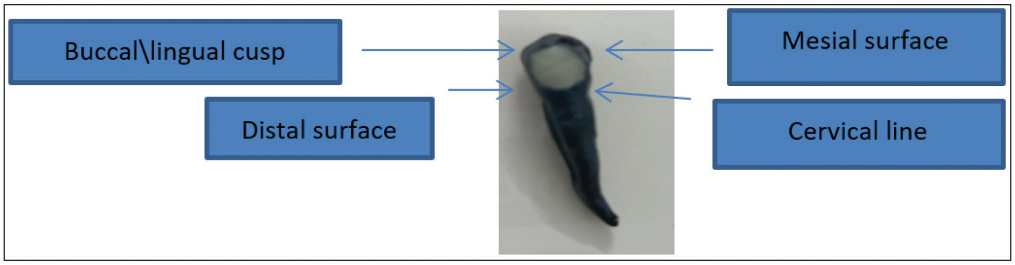

Figure 2: Preparation a window on the buccal surface of the tooth

- Third group: demineralized teeth with indium oxide NP applications.

Preparation of the enamel surface

Each tooth was polished with sandpaper (220 grit: fine), which was manually adjusted, allowing for a smooth surface on each tooth for microhardness testing. Using an orthodontic ruler, a 6-mm-diameter window was standardized on the buccal surface of the teeth. An imaginary line was then drawn from the buccal cusp's tip to the cervical line, and another line was drawn between the mesial and distal tooth surfaces at the points where these surfaces' most prominent curves were present. As a result, the middle portion of each surface was identified. A 6 mm diameter circle of adhesive tape was then cut and applied to the buccal surface of the tooth. Next, the surfaces of the teeth were painted with acid-resistant nail varnish before the adhesive tape was removed, leaving a window there[22] [Figure 2]. For the purpose of assessing the microhardness of teeth, each tooth's root was modified in an acrylic model and covered in red wax up to the cervical line.

Caries-like lesion formation in enamel specimens

The pH cycling approach was used to cause a caries lesion on the enamel surface.[23] Through the manufacture of demineralizing and remineralizing solutions and subsequent pH meter PH adjustment at 37°C, this method was completed [Table 1].

The procedures were performed using the following steps:

1. Microhardness of enamel was measured initially for normal (sound) enamel.
2. Every tooth (in each treatment group) was individually immersed in 20 mL of a demineralizing solution for 7 h at 37°C in an incubator.
3. Specimens were then washed with de-ionized water, and then put in a 20 mL reminerilizing solution for 17 h at 37°C in an incubator.
4. The above-mentioned steps were repeated for 10 days.
5. Using a light microscope to look for any caries-related microscopic alterations that can be seen chalky patch of caries.

Table 1: Demineralization and remineralization solution for pH-cycling

Demineralizing solution (pH = 4.3)	Remineralizing solution (pH = 7)
(0.075) mMol/L acetic acid	(150) mMol/L potassium chloride
(1.0) mMol/L calcium chloride	(1.5) mMol/L calcium nitrate
(2.0) mMol/L potassium phosphate	(0.9) mMol/L potassium phosphate

6. Enamel microhardness was measured after induction of caries lesion.
7. NPs applied on teeth according to the group design; application of indium oxide NPs for 4 min in the treatment group for demineralized enamel and 2 min in the preventative group for natural teeth was followed by a 2 min deionized water rinsing on each tooth. For the next day, at a temperature of 37°C in the incubator, teeth samples were once again preserved in deionized water; this process was repeated every day for a week.
8. Finally, samples were examined again for enamel microhardness after treatment and preventive application with the selected agent.

Microhardness testing

The microhardness measurement was carried out by using a digital micro Vickers hardness tester in the Metal Testing Laboratory, University of Babylon, Faculty of Materials and Ceramics Engineering at a 500 g load for a period of 30 s. For each specimen, three recordings were made for each of these indentations. The mean for these three records was then determined. Vickers microhardness test was done using an optical microscope. Using a diamond indenter with a square base and a 136° included angle between the opposing faces. The magnification employed was ×50 [Figure 3]. The indenter should be applied on a smooth flat surface "facet", and any movement during load use or microhardness measurement, in addition to any over load that may lead to surface crack, should be avoided.[24] After determining the initial microhardness (Hvi, following the pH cycling procedure) and the final microhardness (Hvf, following treatment with the chosen agent), each specimen's alteration difference or change in

Figure 3: (A) Vicker microhardness test. (B and C) Teeth were adjusted in acrylic model and microhardness test

hardness (D) was measured. The mean of this difference (D^-) for each experimental group was then calculated. The ratio of the difference (D^-) to the (Hvi^-), which depicts the rate of change in the specimens' hardness, was also noted for each experimental group.[23,25]

$$(D) = \text{Hvf} - \text{Hvi}$$

$$(D^-) = \sum(D)/n$$

$$(\text{Hvi}^-) = \sum(\text{Hvi})/n$$

% change in hardness values (CH) = $(D^-)/(\text{Hvi}^-) \times 100$

% change of MH in the treatment = 100 * (Rem – Dem)/ Dem.

% change of MH in the preventive = 100 * (Dem – baseline)/ baseline

SMH recovery = 100 * (Rem. – dem)/(baseline – demin.)

Statistical analysis

Statistical Package for Social Science (SPSS version 22.0, Chicago, Illinois) was used to describe, analyze, and present the data. Statistical analyses can be divided into two categories: minimum, maximum, mean, standard deviation (SD), and standard error are used in descriptive analysis for quantitative variables. Shapiro–Wilk test inference involves verifying whether a quantitative variable's distribution is normal. Test the homogeneity of variance for the quantitative variable among groups using the Levene method. Using Dunnett's T3 post hoc test, one way analysis of variance (ANOVA) is used to compare three or more independent groups (equal sample size and unequal variance). Test the differences between two related points using a paired t test, then repeat the measurement using the Bonferroni post hoc test, an ANOVA evaluates the mean differences in K related means between groups. Not significant $P > 0.05$ and highly significant $P < 0.05$ are the levels of significance.

Ethical approval

This study was approved by the Ethical Committee of the College of Dentistry, University of Baghdad (Ref. No. 561 on April 17, 2022).

Results

The characteristics of nanoparticles

Mass concentration of indium nanoparticles

The ablated amounts were 92 µg mL or 3%, with laser energy at 600 mJ and a pulse rate of 125 pulses.

X-ray diffraction pattern (XRD) analysis

Cu K radiation (FEI, Thermo Fisher Scientific) was used to record the X-ray diffraction[26,27], at the University of Kufa, College of Pharmacy for XRD measurement results (1.5406 A). Figure 4 shows the XRD patterns obtained from an InONP suspension solution created by 125 pulses of laser ablation in deionized water. The intensity measurements were calculated in the range of 10°–80°. The produced NPs diffraction peaks at angles of 2 (21.3°, 30.2°, 35.3°, 51.1°, and 60.5°) are reflections from the cubic lattices of the (211), (222), (400), (440), and (622) crystal planes, respectively (JCPDS No. 06-0416) (1.54056), and then use the Scherer equation $D = 0.89/\text{Cos}$ to estimate the grain size. The crystal diameter of the NPs created with 125 pulses was 9 nm.

Field Emission Scanning Electron Microscopy

Field Emission Scanning Electron Microscope (FESEM) of indium oxide NPs prepared by laser was displayed in Figure 5, and field emission scanning electron microscopy (FEI, Thermo Fisher Scientific) provides topographical and elemental information at magnifications of 10× to 300,000°, with virtually unlimited depth of field. A field-emission cathode in the electron gun of a scanning electron microscope provides narrower probing beams at low as well as high electron energy; the resulted NPs were almost spherical in shape and homogeneous.

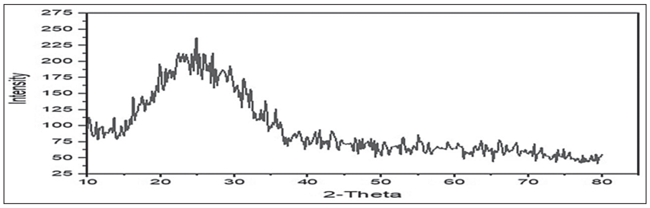

Figure 4: XRD of indium oxide NPs by PLAL laser (125pulses)

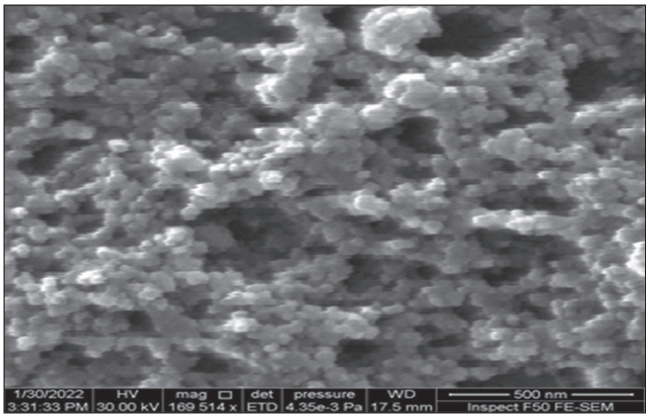

Figure 5: Field emission scanning micrograph of indium oxide NPs prepared by laser ablation at 125 laser pulses

Ultraviolet-visible absorption spectrum

Optical properties spectrum of the indium oxide NPs using UV-visible spectrophotometer was used, with a double beam in the range of (200–800 nm) for NPs sample. These calculations of the resulted nanoparticles colloidal solution at room temperature inside quartz cell having optical path of 1 cm. Optical absorption of colloidal for InONPs sample prepared at 125 laser pulses and energy of 600 mJ is depicted in Figure 6. For the produced sample, the absorption spectra contained peak was 290 nm.

The effect of indium oxide nanoparticles on human dental enamel *in vitro*

Microhardness test

Using the Shapiro–Wilk test at $P > 0.05$, Table 2 demonstrates that surface microhardness is regularly distributed throughout phases, stages, and groups (with statistically no significant differences). Table 3 shows descriptive and statistical analyses of surface microhardness among phases, groups, and stages. The mean value of microhardness (change in microhardness) of the InONPs and NaF groups was reported to have increased during the preventive phase, with a statistically highly significant difference ($P = 0.001$), but the mean value of the InONPs microhardness group had been higher than the mean value of the NaF group. It was reported no significant differences in microhardness in the control group (DW) ($P > 0.05$).

According to treatment phase, it was shown a marked decrease in microhardness in demineralization stage in control (DW), InONPs and NaF groups with statistically highly significant

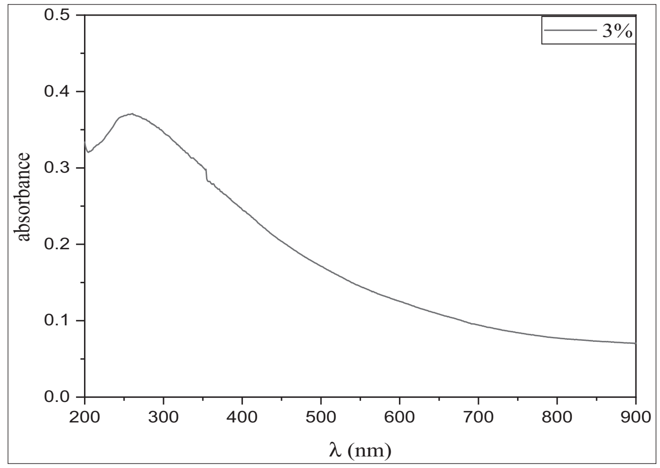

Figure 6: UV-visible absorption spectra of InONPs suspension that was prepared by pulsed laser ablation in liquid

differences ($P < 0.001$), whereas there was no significant difference after remineralization in control group (DW) ($P > 0.05$), but there were highly significant differences after remineralization by InONPs and NaF groups ($P < 0.001$). A = paired T test, b = repeated measure ANOVA (F), in the baseline, the intercomparisons in the preventive and treatment phases between DW, indium oxide NPs and 0.05% NAF, there is no significant difference between them, which are $F = 2.528$, P value = 0.099, NS and $F = 1.227$, P value = 0.309, NS, respectively. The intercomaprisons for DW, indium oxide nano and 0.05% NAF between preventive and treatment phases are $F = 0.124$, P value = 0.729, NS, $F = 2.11$, P value = 0.052, NS, and $F = 2.13$, P value = 0.051, NS, respectively, and in the demineralization phase, there is no significant difference between groups ($F = 1.019$, P value = 0.375, NS. Multiple pairwise comparisons of surface microhardness between phases by groups in the treatment using Bonferoni post hoc are shown in Table 4. Highly significant differences ($P < 0.001$) except in control group between demineralization and remineralization no significant difference were recorded ($P > 0.05$). Descriptive and statistical tests of remineralization among groups and phases are shown in Table 5. Highly significant difference was recorded for microhardness in all groups for preventive and treatment ($P < 0.001$). As in Table 6, multiple pairwise comparison of surface microhardness among groups by phases using Bonferroni posthoc, it was showed in preventive phase, highly significant differences between control negative (deionized water) and InONPs groups but not significant differences between InONPs and NaF groups (control positive). In treatment phase, it was found highly significant differences between InONPs and control groups, and the same result was reported between InONPs and NaF groups.

Changes in microhardness of enamel surfaces in preventive and treatment phases

Changes in microhardness values after the preventive phase, treatment phase, preventive and treatment are

Table 2: Surface microhardness among phases, stages, and groups using the Shapiro–Wilk test

Phases	Stages	Groups	Shapiro–Wilk		
			Statistic	df	*P* value
Baseline	Preventive	DW	0.962	10	0.812
		Indium oxide nano	0.952	10	0.698
		0.05% NAF	0.895	10	0.191
	Treatment	DW	0.915	10	0.318
		Indium oxide nano	0.935	10	0.497
		0.05% NAF	0.869	10	0.098
Demineralization	Preventive	DW	–		
		Indium oxide nano			
	Treatment	DW	0.916	10	0.326
		Indium oxide nano	0.946	10	0.625
		0.05% NAF	0.924	10	0.392
Remineralization	Preventive	DW	0.969	10	0.879
		Indium oxide nano	0.938	10	0.529
		0.05% NAF	0.903	10	0.238
	Treatment	DW	0.919	10	0.352
		Indium oxide nano	0.917	10	0.333
		0.05% NAF	0.944	10	0.596

df = degree of freedom, $P > 0.05$ (not significant)

Table 3: Descriptive and statistical tests of surface microhardness among phases, groups, and stages

Phases	Groups		Baseline	Demineralization	Remineralization	Statistics	*P* value	%MH	MHRec
Preventive[a]	Deionized water	Mean	358.670		359.147			0.133	
		SD	11.974		12.057			0.197	
	Indium oxide nanoparticles	Mean	366.830		393.950	17.402	0.000000	7.419	
		SD	5.604		3.043			2.023	
	0.05% NAF	Mean	365.470		383.200	16.307	0.00001	4.866	
		SD	7.212		4.861			0.963	
Treatment[b]	Deionized water	Mean	354.940	130.320	131.620	159.778	0.000	1.140	0.551
		SD	31.265	24.743	23.962			1.077	0.395
	Indium oxide nanoparticles	Mean	344.390	153.710	229.150	258.341	0.000	54.441	41.228
		SD	12.999	42.942	40.199			25.660	10.445
	0.05% NAF	Mean	340.380	143.040	180.430	162.948	0.000	27.672	20.292
		SD	15.379	39.774	48.086			17.089	13.546

%MH = change in microhardness, MHRec = microhardness recovery

Table 4: Multiple pairwise comparisons of surface microhardness between phases by groups in the treatment using Bonferroni post hoc

Groups	Phases		Mean difference	*P* value
Deionized water	Baseline	Demineralization	224.620	0.000
		Remineralization	223.320	0.000
	Demineralization	Remineralization	–1.300	0.999
Indium oxide nanoparticles	Baseline	Demineralization	190.680	0.000
		Remineralization	115.240	0.000
	Demineralization	Remineralization	–75.440	0.000
0.0 5% NAF	Baseline	Demineralization	197.340	0.000
		Remineralization	159.950	0.000
	Demineralization	Remineralization	–37.390	0.000

shown in Figures 7–9, which are estimated by special equations. Values from these figures reflect a very minor changes in the microhardness for the controls (teeth in deionized water) for both preventive and treatment group. Marked changes in microhardness values after NPs and sodium fluoride applications for both preventive

Table 5: Descriptive and statistical tests of remineralization among groups and phases

Groups		Phases		*F*	*P* value
		Preventive	Treatment		
Deionized water	Mean	359.147	131.620	331.802	0.000
	SD	12.057	23.962		
Indium oxide nanoparticles	Mean	393.950	229.150	174.071	0.000
	SD	3.043	40.199		
0.05% NAF	Mean	383.200	180.430	263.524	0.000
	SD	4.861	48.086		
F		4.071	30.484		
P value		0.023	0.000		

Table 6: Multiple pairwise comparison of Surface microhardness among groups by phases after treatment using Bonferroni post hoc

Phases	Groups		Mean difference	P value
Preventive	Deionized water	Indium oxide nanoparticles	–34.803	**0.007**
		0.05% NAF	–24.053	**0.048**
	Indium oxide nanoparticles	0.05% NAF	10.750	0.393
Treatment	Deionized water	Indium oxide nanoparticles	–97.530	**0.000**
		0.05% NAF	–48.810	**0.000**
	Indium oxide nanoparticles	0.05% NAF	48.720	**0.000**

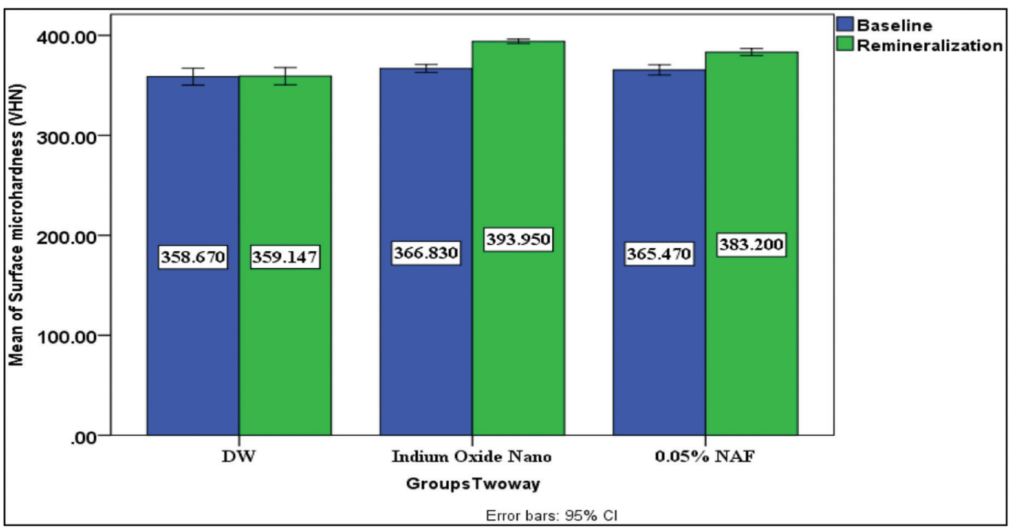

Figure 7: Changes in microhardness values in preventive stage

and treatment groups were noted. No difference was marked in microhardness between InONPs and NaF in preventive phase and marked increase in microhardness in treatment phase after both treatments with InONPs and NaF applications, but the effect was higher in InONPs than NaF.

Discussion

The characteristics of indium oxide NPs suspension prepared by pulse laser ablation

The preparation method of indium oxide NPs suspension by laser ablation was followed in this study, but with different pulses, it was also used by previous Iraqi studies.[20,28,29] Pulsed laser ablation in liquid is a clean, straightforward chemical process that yields a high-purity end product.[9,10] With fixed laser energy, indium oxide NPs were created. Because of the laser-plume shoot technique, during the laser pulse-attached target surface, an amount of ablation can be seen and a sound can be heard.[11] By estimating the change in mass concentration between before and after laser ablation, the amount of NPs in the suspension solution that have been ablated increases with increasing laser pulses.[17] This laser pulses interact with the bulk of the material, allowing for more heat transfer

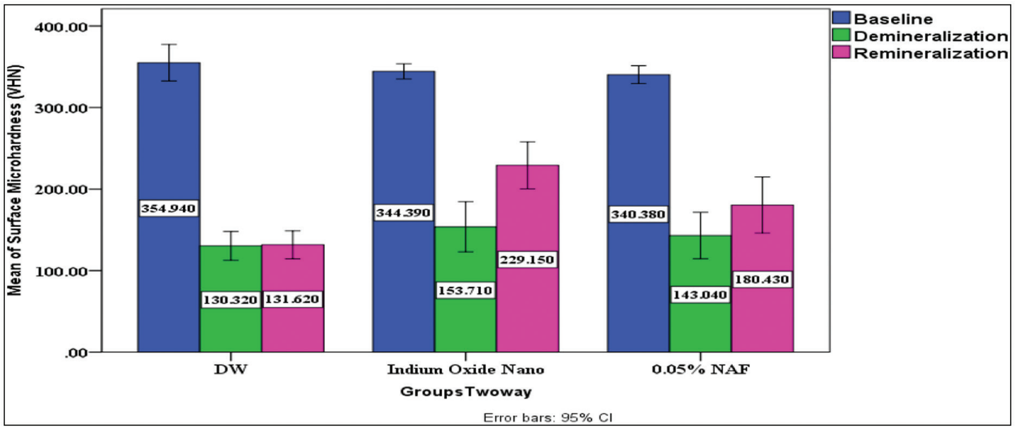

Figure 8: Changes of surface microhardness values in treatment stage

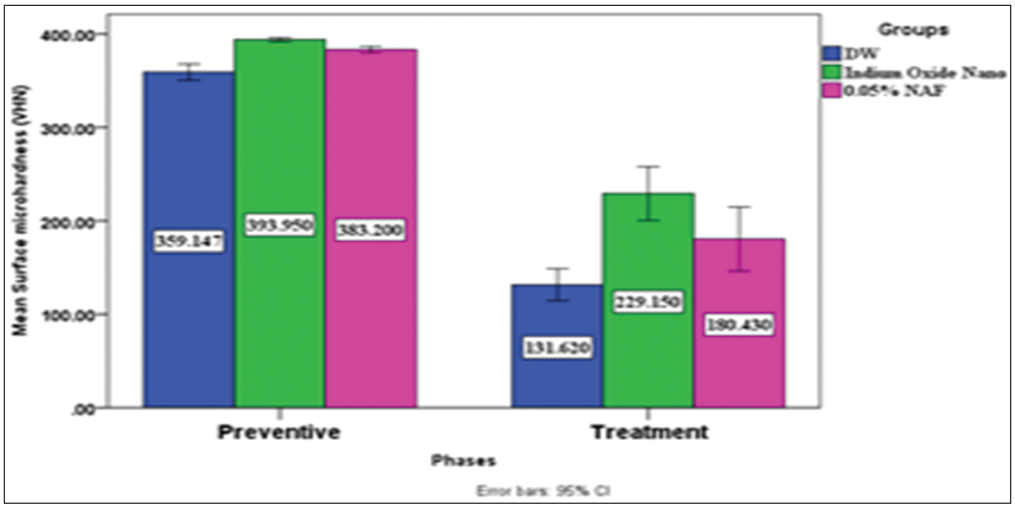

Figure 9: Changes in microhardness in preventive and treatment between control and InONPs

to the target, causing a significant amount of surface target to evaporate.[30] Field emission scanning micrograph displayed the resulted NPs, which were almost spherical in shape and homogeneous, and this was confirmed by previous study.[31,32] The absorption spectra having a peak (290) nm of InONPS were prepared with UV absorption spectroscopy.

The effect of indium oxide nanoparticles on human dental enamel *in vitro*

This study investigated *in vitro* the effects of InONPs on sound and demineralized enamel, whereas no previous study investigated about InONPs suspension solution application on human dental enamel *in vitro*. The integrity of dental hard tissue is assessed in part by measuring the surface microhardness (SMH) of human enamel. It frequently serves as a stand-in indicator of the "healthiness" of the enamel surface. Healthy or remineralized tissue is harder than acid-challenged or demineralized tissue, which tends to be softer. When utilized to examine the integrity of enamel during *in situ* remineralization/demineralization clinical testing, SMH testing is frequently employed as the primary measure during *in vitro* pH cycling testing. This was verified by the Vickers method, which had positive outcomes in earlier experiments.[22,33]

The current result showed that increase in the microhardness of sound enamel after InONPs application (preventive effect) with statistically highly significant difference. As such, there were no previous studies to compare the result of the current study. The explanation of this was due to incorporation of this metal indium ions within the structure of the enamel; however, the mechanism of indium incorporation is not understood. A metal nanoparticles have ability to stick to hydroxyl apatite crystal was discovered, and an increase in surface area made it easier for them to form a mechanical bond with dental tissues, which was verified by research.[4] According to a prior study,[34] the small size and spherical shape of the metal NPs boost the contact surfaces in addition to their antimicrobial properties, which work as a preventative measure against the initiation of caries. Metal NPs protective function could be attributed to their extremely small particle size, which made it easier for the material to penetrate the enamel structure and have a greater impact.[35]

The present study showed application of InONPs on demineralized enamel after pH cycling procedure was applied on sound enamel for carious lesion initiation,[23] which was also used by further Iraqi studies.[22,33] This method of pH cycling is simple, reliable, and very successful in the initiation of dental caries, also is considered to be a better simulation of the *in vivo* situation, in which the enamel subjected to de- and remineralizing sequences. The initiation of carious lesion in the current study was conducted in ten days.

After pH cycling, a statistically highly significant decrease in the microhardness of the enamel surface was discovered as a sign of enamel demineralization and the beginning of a carious lesion. This is because any drop in the environment's pH below the critical pH (5.5) results in the formation of an acidic medium that causes the minerals of teeth, primarily calcium and phosphorus, to move outward, leaving behind micropores and lowering hardness.[36]

Regarding the demineralized group treated with InONPs, the mean values of microhardness were increased as compared to the demineralization stage with a statistically highly significant difference. No previous study regarded the remineralization (treatment) potential of indium oxide NPs. The possible explanation was attributed to that incorporation of indium metal ions in the structure of tooth enamel; however, the mechanism of their acquisition is still not fully understood, but their incorporation results in lower relative content of carbonate (in which carbonate is positively associated with dental caries and lower microhardness), so this will positively affect the physical and chemical properties of enamel.[23] Furthermore, NPs have been shown to bind to hydroxyapatite surfaces and may therefore be consumed in the outer parts of the lesion rather than penetrating into deeper parts.[37] This is supported by a prior Iraqi study conducted by Albazaz in 2017 using metal iron oxide NPs for enamel remineralization.[33]

Using different fluorides can help prevent cavities or demineralization resistance from occurring in the first step. One effective anti-caries agent among those on the market is fluoridated mouthwash. The use of it would significantly lessen tooth decay. Since the 1930s, fluoride has been used extensively to prevent dental caries[38]; however, the impact of other components on dental caries is not well supported. The effectiveness of sodium fluoride in remineralizing the initial carious lesion and boosting resistance to caries is well established from experimental research.[39,40] Because of these disadvantages, as fluorosis and toxicity,[40] it may be beneficial to look for alternatives through scientific research. Another effective anti-caries drug that combines preventative and antibacterial qualities is indium oxide NPs.

It was reported that the mean values of microhardness of InONPs were higher than mean values of sodium fluoride mouth wash with no significant difference in preventive phase and highly significant differences in treatment phase. Because of small size, large surface area, and antimicrobial activities of metal NPs[41] with highly significant difference between silver NPs and sodium fluoride, but Teixeira et al.[42] and El-Desouky et al.[35] were reported no significant differences between silver NPs and sodium fluoride effects on enamel microhardness. Because of small size, large surface area, and antimicrobial activities of metal NPs[41] with highly significant difference between silver NPs and sodium fluoride, but Teixeira *et al*.[42] and El-Desouky *et al*.[35] were reported no significant differences between silver NPs and sodium fluoride effects on enamel microhardness. Metal NPs have difficulties because it is unclear how they cause remineralization *in vitro*; it could be due to their size or stability. The hydroxyl group in hydroxyapatite is replaced by a fluoride group, which is fluoride's well-known mode of action hydroxyapatite crystals (HAP). As a result, the density of HAP crystals rises, making enamel less vulnerable to further acid damage.

Remineralization from this method, however, may only be partial in conditions where cavities or severe demineralization are already present because there are hardly any undamaged HAP crystal sites. Additionally, different areas of the HAP crystal than fluoride could be coated by metal NPs.[41] Remineralization systems can be further enhanced with more study, opening the door to better and more sophisticated strategies for caries and demineralization prevention. Although the mean microhardness values of dental demineralized enamel were raised by the InONPs suspension solution, none of these values came close to the original microhardness values of the healthy teeth. This can be a result of the experiment's timing of application. For a period of seven days, teeth were treated for four minutes per day. More research will be required in the future to discover whether the microhardness reaches the original value or is elevated more. Increasing the length of treatments to weeks rather than one week may increase the microhardness values. InONPs suspension solution needs to be confirmed by additional studies (experimental as well as human studies on an artificial as well as a natural carious lesion) before any recommendation of using InONPs in the dental practice is made, despite there being no significant difference between InONPs and NaF in the preventive phase and a significant difference of InONPs higher than NaF in the treatment phase.

Conclusion

Increase in microhardness was recorded for both preventive and treatment groups after indium oxide NPs application and there is high mean difference in compared to control, from that this NPs is suggested to be alternative to sodium fluoride.

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Conflicts of interest

There are no conflicts of interest.

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