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Improvement of Human Dental Enamel Using Laser-Prepared Indium Oxide Nanoparticles Suspension Solution (*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, but new and innovative nanomaterials approaches are transforming the industry. A variety of unique nanomaterials are now easily available to assist remineralization of dental enamel surface.
Objective: investigate the effect of indium oxide nanoparticles application in comparison to de-ionized water and sodium fluoride on human tooth enamel by assessing the morphological changes in enamel ultra-structure. Using a field emission scanning electron microscope, x-ray diffraction, and ultraviolet visible absorption to evaluate the physical characteristics of indium oxide nanoparticles created by laser ablation in liquid.
Method: Teeth samples were seventy-two divided randomly into two phases; each phase consists of study and treated groups with indium oxide nanoparticles, control positive treated with sodium fluoride, and control negative with deionized water. Each group was consisted of twelve teeth, from each group two teeth for scanning electron microscopy and the rests were for energy dispersion spectroscopy. **Result:** result showed change in chemical composition of dental enamel was recorded for both preventive and treatment groups after nanoparticles application and this was supported by energy dispersive spectroscopy and scanning electron microscope.
Conclusion: Laser prepared indium oxide nanoparticles colloidal suspensions were found to be effective remineralizing agent to dental enamel and thus can be considered as an alternative to sodium fluoride mouth wash.
Keywords: Indium oxide nanoparticles, human normal dental enamel, scanning electron microscopy, energy dispersion spectroscopy.

INTRODUCTION
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, 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. This engineering specialty used molecular machines with precise structures that are equivalent to nano means 10^{-9}, because of their distinctive properties, such as their better surface-to-volume ratio and enhanced bioavailability toward cells and tissues [(2,3)]. Due to metal nanomaterial's ability to disrupt bacterial metabolism and inhibit biofilm development, significant antibacterial action was displayed by metal and 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 was used in solar cells, photocatalysts, organic light-emitting diodes, architectural glasses, 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)]. Since the demand for consumer electronics has increased, so has occupational exposure to particles containing indium [(7,8)]. 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 nanoparticles have been created using a variety of methods, including chemical, solvo/hydrothermal, pulsed laser deposition, and others [(11,12,13)]. The Gram negative bacterium *Escherichia coli* (E. coli) were killed by the metal oxide indium oxide nanoparticles, which has been shown to have antibacterial action. This metal oxide also had therapeutic potential for the treatment of infectious disorders [(14)].

With a wet chemical process, novel indium and titanium oxide nanoparticles with antibacterial action against Gram-positive pathogens were created. Gram positive *Staphylococcus aureus* and Gram negative *E. coli* were both effectively inhibited by indium oxide nanoparticles that were created using the hydrothermal technique [(15)]. The acquired results showed that the pathogenic *Candida albicans* might be inhibited *in-vitro*

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by deformation and distortion of the cell wall and membrane of Candida cells, which may result in cell death [16]. To date no foreign study was conducted to investigate the improvement of human dental enamel using laser prepared indium oxide nanoparticles 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 duration of about 10 ns. The formed colloidal solution has been collected after the nanoparticles were produced using fixed laser energy and the number of pulses with 125 pulses [11].

Using a sensitive electronic scale, the target was weighed both before and after the ablation technique to determine the concentration of colloid [17]. The FESEM images were used to determine the form of the nanoparticles. When the suspension of the produced NPs was deposited onto glass slides and allowed to dry, an X-ray was used to characterize them. Cu-K (1.54eV) 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 nanoparticles were measured using a double-beam ultraviolet-visible spectrophotometer at wavelengths between 190 and 1100 nm [20].

Teeth samples

At least 72 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, 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. Each tooth surface was examined under good lighting to check for any cracks or defects on the enamel surface. 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, and then the cup was placed in a refrigerator to prevent tooth dehydration. Following that, teeth samples were stored in a refrigerator at 4°C until use [21].

Study design

Study design was involved (72) teeth which divided randomly into two phases, each phase with 3 groups, each group which was consisted of ten teeth for energy dispersion spectroscopy and two teeth for SEM examination.

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 NaF0.05% mouthwash application (positive control).
- Third group: sound teeth with nanoparticles 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).
- Third group: demineralized teeth with nanoparticle applications.

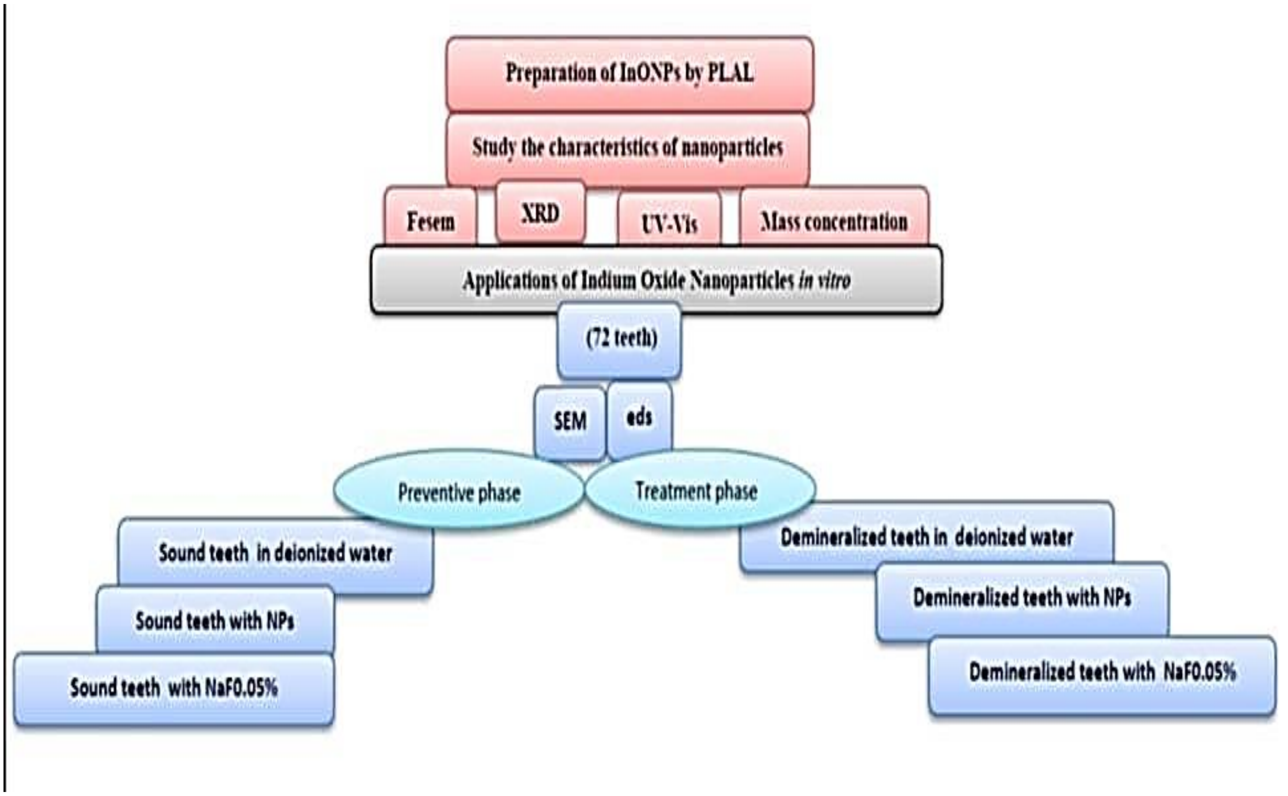

Figure (1): Design of the study

Preparation of enamel surface

Each tooth was measured 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 6mm 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): Preparation a window on the buccal surface of the tooth.

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(2.2).

Table (1): Demineralization and remineralization solution for PH-cycling.

Demineralizing solution(pH=4.3)	Remineralizing solution(PH=7)
(0.075) Mol/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

Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (EDS)

A concentrated electron beam was utilized in SEM to scan the enamel surface and investigate the morphological changes that occur. The interactions between electrons and atoms resulted in a variety of signals that contain details on the topography and make-up of each sample surface. An image was produced by combining the position of the electron beam with the detected data, which was typically done in a raster scan pattern. Two representative specimens from each group were chosen to create the SEM sample. The chosen teeth were gold coated by putting them in a vacuum system coating equipment to help or improve the imaging of samples (ion sputter).

A conductive coating of metal formed on the sample as a result, which prevents charging, reduces heat damage, and enhances the secondary electron signal needed for topographic analysis in the SEM. The specimens had been coated in gold and were prepared for SEM analysis [24].Utilizing EDS, it was possible to determine the weight percentages (wt %) of calcium (Ca), phosphorous (P), and oxygen (O) as part of an elemental analytical inquiry or chemical characterization of a substance. It depends on the interactions between an X-ray excitation source and the sample being studied. The most fundamental spectroscopic assumption, that each element had a unique atomic structure allowing for a distinctive collection of peaks on its electromagnetic emission spectrum, contributes to a significant portion of its ability to characterize materials [25].

Ethical approval: This study was approved by the Ethical Committee of the College of Dentistry/ University of Baghdad (Ref. No. 561 in 17 /4/2022).

Statistical analysis

Statistical Package for Social Science (SPSS version -22, Chicago, Illinois, USA) 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 were used in descriptive analysis for quantitative variables. basic, cluster chart bar, and line bar graphs. Shapiro-Wilk test inference: Verify that a quantitative variable's distribution is normal. Test the homogeneity of variance for the quantitative variable among groups using the Levene method. Comparing the mean differences between groups that have been divided based on three independent variables, the general linear model (Factorial Analysis Of Variance) checks to see if there is an interaction between those variables and the dependent variable. Using Dunnett's T3 posthoc 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 posthoc test, an ANOVA evaluates the mean differences in K related means between groups. Not significant $P>0.05$, higly significant $P<0.05$ are the levels of significance.

RESULTS

The Characteristics of nanoparticles

Mass concentration of indium nanoparticles

The ablated amounts were (92) µ g|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 was used to record the x-ray diffraction results (1.5406 A). Figure (3) showed 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 to 80 degrees. The produced nanoparticles' 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/Cos to estimate the grain size. The crystal diameter of the nanoparticles created with 125 pulses was 9nm.

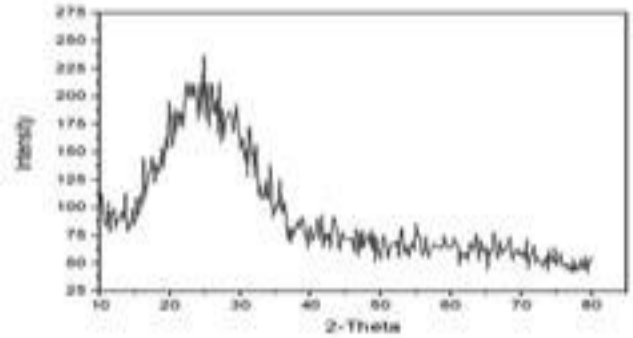

Figure (3) XRD of indium oxide nanoparticles by PLAL laser(125pulses).

Field Emission Scanning Electron Microscope (FESEM)

Field Emission Scanning Electron Microscope of indium oxide NPs prepared by laser was displayed in figure (4), the resulted nanoparticles almost spherical in shapes and homogeneous.

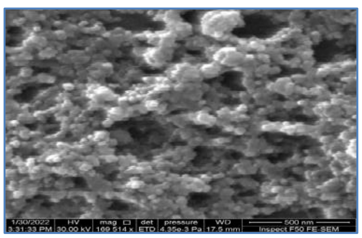

Figure (4): FESEM of indium oxide NPs prepared by laser ablation at 125 laser pulses.

UV-Visible Absorption Spectrum

InONPs suspension produced by pulsed laser ablation in liquid, in which was calculated to have UV-visible absorption spectra spanning the wavelength range of (200-1100nm). Optical absorption of colloidal for InONPs sample prepared at 125 laser pulses and energy 600mJ is depicted in figure (5). For produced sample, the absorption spectra contain peak was (290) nm.

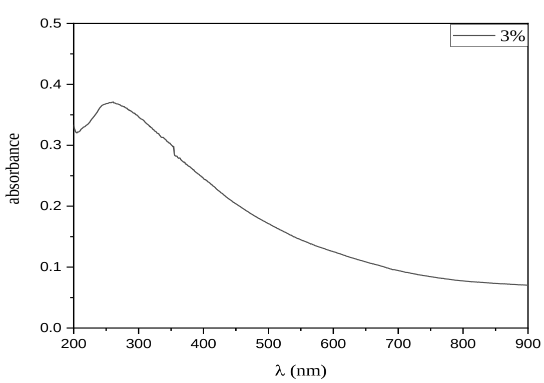

Figure (5): UV- visible absorption spectra of InONPs suspension that prepared by pulsed laser ablation in liquid.

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

Energy dispersion spectroscopy

For all groups regarding the atomic percentage of oxygen (O), calcium (Ca) and phosphorous (P). Results above show that all results of EDX are normally distributed using: Shapiro Wilk test at p>0.05 as seen in table (2).

According to Oxygen, results revealed that mean value of oxygen atoms decreased after nanoparticles applied on sound tooth. In comparison with sound group, there was high percentage of oxygen atoms in demineralization group. There was reduction in oxygen atoms after application of nanoparticles on demineralization group in comparison to demineralization group. ANOVA test showed statistically highly significant difference, as seen in table (3) below. Mean differences of oxygen atoms between groups illustrated in table (4). It was found highly significant mean differences between all groups except not significance differences between sound group and demineralization and nanoparticles groups. Estimated marginal mean of oxygen atoms showed in figure (6). It was found the highest percentage of oxygen atoms was in demineralization group then demineralization-nanoparticles group, sound group and sound-nanoparticles group respectively. It was found that the mean value of calcium atom in demineralization group less than sound group, ANOVA test showed highly significant difference, as seen in table (5).Mean difference of calcium illustrated in table (6). It was found highly significant differences among all groups except mean difference between sound and sound-NPs in addition to demineralization group and demineralization-NPs group there was no significant difference. Figure (7) showed estimated marginal means of calcium. It was found the highest mean value was in sound group, then there was reduction in mean values of sound- NPs, demineralization and demineralization- NPs groups respectively. According to phosphorous atoms, the result showed that the mean value of phosphorous atoms decreased after nanoparticles application on sound enamel. In comparison to sound group, the demineralization group was with less phosphorous atoms. It was found decrease in phosphorous atoms after application of nanoparticles on demineralization group. ANOVA test showed highly significant difference, as seen in table (7). Mean difference of phosphorous atoms among groups in table (8). The result showed the highly significant mean difference between all groups. Estimated marginal means of phosphorous in figure (8). The result showed the highest mean value was in sound group, then it was found less mean value in sound-NPs, demineralization and demineralization–NPs respectively.

Table (2): EDX distribution using Shapiro Wilk test.

Regions	Oxygen			Calcium			Phosphorous		
	Statistics	df	p	Statistics	df	p	Statistics	df	p
Sound	0.878	10	0.124	0.869	10	0.098	0.926	10	0.410
Sound+NPs	0.895	10	0.191	0.855	10	0.066	0.942	10	0.575
Demineralization+NPs	0.924	10	0.393	0.928	10	0.426	0.872	10	0.106

df= degree of freedom , P= p value.

Table (3): Descriptive and statistical test of oxygen.

	Sound	**Sound +NPs**	**Demineralization**	**Demineralization+NPs**
Minimum	55.900	53.800	62.100	58.000
Maximum	62.100	60.000	70.900	62.100
Mean	59.910	57.320	64.630	60.030
±SD	1.885	1.872	2.708	1.334
±SE	0.596	0.592	0.856	0.422
F	20.626			
P value	**0.001**			
Effect size	0.898			

Table (4): Pairwise Comparisons of oxygen among groups using Bonferroni

EDX		Mean difference	p value
Sound	Sound+NPs	2.590	**0.00006**
	Demineralization	-4.720	**0.00109**
	Demineralization+NPs	-0.120	1.00000
Sound+NPs	Demineralization	-7.310	**0.00023**
	Demineralization+NPs	-2.710	**0.00311**
Demineralization	Demineralization+NPs	4.600	**0.00114**

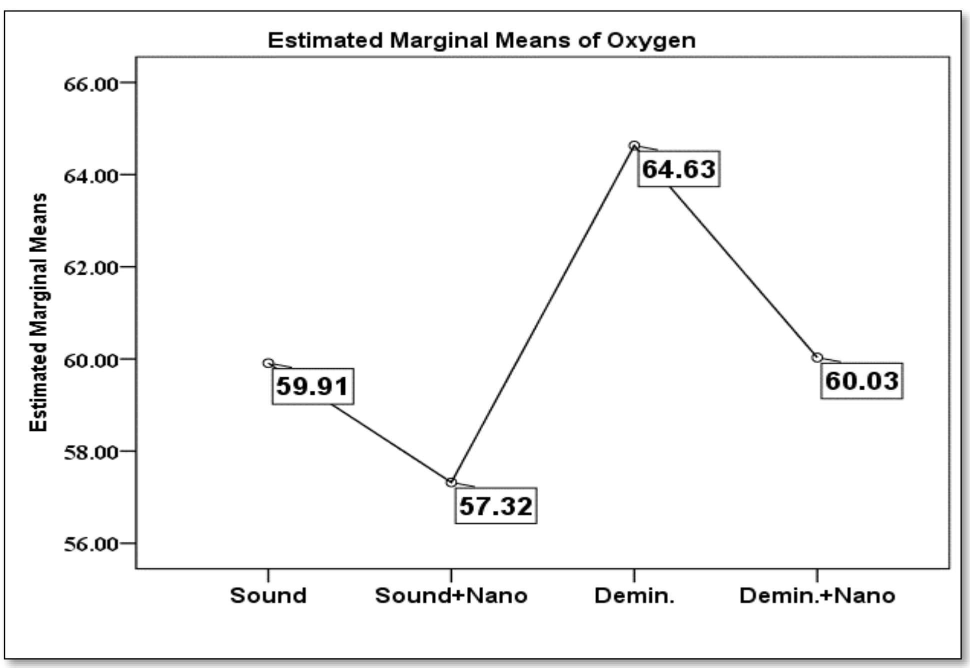

Figure (6): Estimated marginal mean of oxygen.

Table (5): Descriptive and statistical test of Calcium

	Sound	Sound+NPs	Demineralization	Demineralization+NPs
Minimum	12.900	12.600	10.100	10.000
Maximum	29.200	28.900	20.200	20.000
Mean	23.550	23.100	16.510	16.280
±SD	5.191	5.226	2.929	3.036
±SE	1.642	1.653	0.926	0.960
F	20.043			
P value	**0.001**			
Effect size	0.896			

Table (6): Pairwise Comparisons of Calcium among groups using Bonferroni

EDX		**Mean difference**	**p value**
Sound	**Sound+NPs**	**0.190**	**0.58383**
	Deminralization	**7.040**	**0.00041**
	Deminralization+NPs	**7.270**	**0.00041**
Sound+NPs	**Deminralization**	**6.850**	**0.00051**
	Deminralization+NPs	**7.080**	**0.00050**
Demineralization	**Deminralization+NPs**	**0.230**	**0.59023**

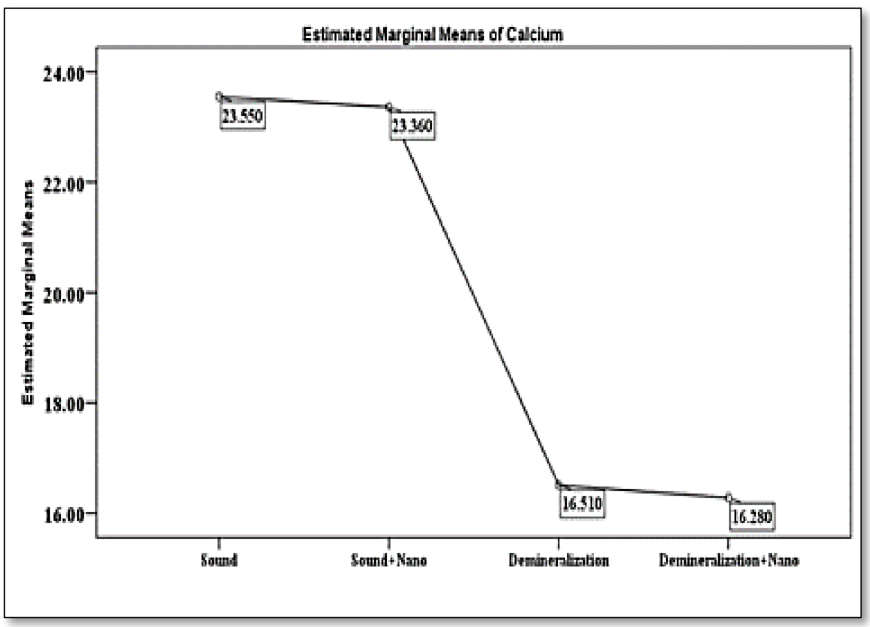

Figure (7): Estimated marginal means of calcium.

Table (7): Descriptive and statistical test of Phosphorous

	Sound	Sound+NPs	Demineralization	Demineralization+NPs
Minimum	10.300	9.500	8.100	7.000
Maximum	18.800	17.200	13.900	11.300
Mean	15.060	13.780	11.660	9.610
±SD	2.196	2.055	1.718	1.468
±SE	0.694	0.650	0.543	0.464
F	60.941			
P value	**0.000022**			
Effect size	0.963			

Table (8): Pairwise Comparisons of phosphorous among groups using Bonferroni

EDX		Mean difference	p value
Sound	Sound+NPs	1.280	**0.00000**
	Deminralization	3.400	**0.00001**
	Demineralization+NPs	5.450	**0.00000**
Sound+NPs	Demineralization	2.120	**0.00008**
	Demineralization+NPs	4.170	**0.00000**
Demineralization	Demineralization+NPs	2.050	**0.00000**

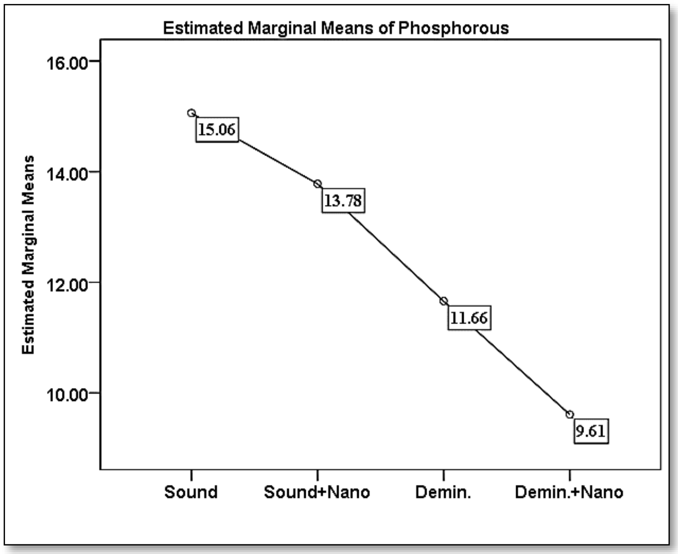

Figure (8): Estimated marginal means of phosphorous atoms among groups.

Microscopic feature of the outer enamel surface using scanning electron microscope (SEM):

The structural changes for the surface of enamel were presented at the following figures for each group. For the sound enamel surface, normal, smooth, intact surface without any alteration and some holes can be seen, figure (9). According to normal enamel surface after indium oxide nanoparticles applications, it was showed deposition of nanoparticles with translucent crystals structure as layer that cover the enamel surface that homogenously distribution, figure (10).

Figure (11) showed sound enamel after sodium fluoride application. Sodium fluoride act as globular layer adhere on the enamel surface. The spherical globular agglomerates were observed varies in sizes from place to place. For the demineralized enamel surface, the structure of the enamel surface was changed in which it lost normal feature with irregular prisms. It was showed numerous voids with, micropores seen on the enamel surface, figure (12).

For demineralized enamel after nanoparticles application, the deposition of NPs over demineralized enamel were translucent smooth and uniform that, occlude the micropores created after demineralization, figure (13). Figure (14): showed demineralized enamel after sodium fluoride application. Globules were spherical with different sizes that occludes the micro pores that's formed after demineralization.

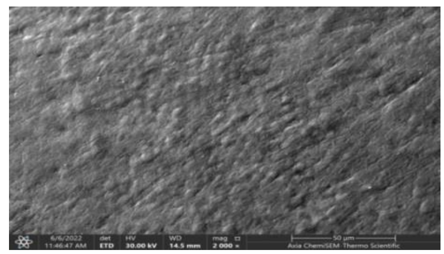

Figure (9): SEM for normal smooth enamel surface at different magnification.

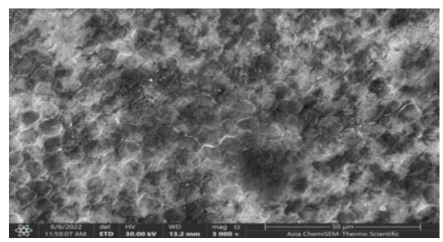

Figure (10): SEM for sound enamel surface after InO nanoparticles applications. The figure shows distributed of NPs on the enamel surface.

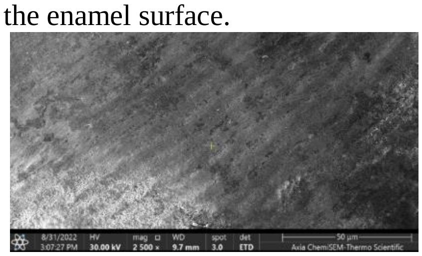

Figure (11): sound enamel after sodium fluoride application.

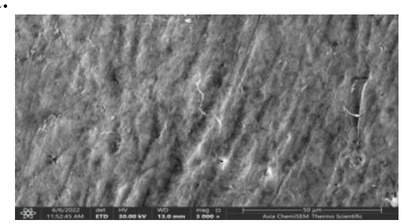

Figure (12): SEM for enamel surface after demineralization. Arrow showed micropores presented.

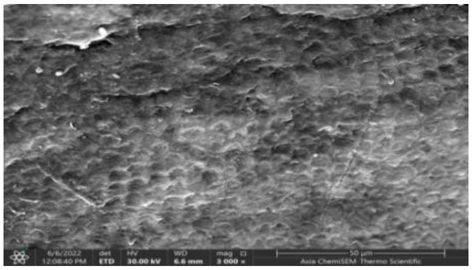

Figure (13): SEM for demineralized enamel surface after application of NPs. NPs covered the micro pores and fill the defects.

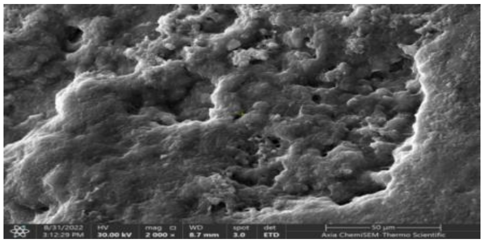

Figure (14): Demineralized enamel after sodium fluoride application.

DISCUSSION

The Characteristics of Indium oxide nanoparticles suspension prepared by pulse laser ablation:

The preparation method of indium oxide nanoparticles suspension by laser ablation (Acacia et al, 2010) was followed in this study, but with different pulses, which was also used by further previous Iraqi studies [26, 27, 20]. Pulsed laser ablation in liquid is presently viewed as an appealing technique for producing nanomaterials since it is a clean, straightforward chemical process that yields a high-purity end product. By adjusting the pulse duration or the laser's energy, colloidal NPs allow for fine-tuning of the size distribution. Additionally, the technique works at standard pressure and temperature [9,10].

With fixed laser energy, indium oxide nanoparticles were created. Due to 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 result is explained by how a succession of laser pulses interacts with the bulk of the material, allowing for more heat transfer to the target, boosting its surface temperature and causing a significant amount of surface target to evaporate [28]. Additional study from Iraq support this [20] .Cu K radiation (1.5406 A) was used to capture the x-ray diffraction data, which was confirmed by study conducted in Iraq [15] and other study conducted previously [16] . With standard indexing at the JCPDS standards (Card No. 06-0416), the XRD spectrum of In2O3 showed peaks with diffraction pattern patterns that are correlated with the bcc (body-centered cubic) structure of In_2O_3, and the lattice constant is close to its value. Using that information, the Scherer equation was applied to determine the grain size for just that peak. FESEM micrograph displayed the resulted nanoparticles, they were almost spherical in shape and homogeneous, this confirmed by previous study [29, 30].The absorption spectra have peak (290) nm of InONPS were prepared with UV absorption spectroscopy. This absorption spectra peak of this study was nearly the same of previous study [15]. Amount of NPs in the colloidal increases with increasing laser pulses [20]. The accumulation of nanoparticles that remain in the liquid surrounding the target, creating what is known as a colloidal solution, was found to cause an increase in absorbance. This interaction with laser radiation is prolonged through the process of free electrons absorbing its energy, which lowers the intensity of the incident laser light [28].

The effect of Indium Oxide Nanoparticles on human dental enamel *in vitro*

This study was investigated *in vitro* the effects of InONPs on sound and demineralized enamel, in which no previous study about InONPs suspension solution application on human dental enamel in vitro. 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 [22, 31].

The finding of EDS analysis showed no increase in calcium and phosphorous of sound enamel surface after application of indium oxide nanoparticles, as there was no source of free calcium and phosphorous ions to redeposit on dental enamel surface. There was marked reduction in phosphorous ions this may be substitution occurred between phosphorous ions and indium ions, however this require further future study for more explanation. The SEM micrograph gave a sure for the findings, in which the deposition of InONPs over sound enamel was with translucent crystals structure as a protective layer on the enamel surface. 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. It was found the ability of metal nanoparticle to adhere to hydroxyl apatite crystal, the increase surface facilitates their mechanical bond with dental tissues, this confirmed by previous study [32], in which the small size of the metal nanoparticles and its spherical shape increases the contact surfaces in addition to its antimicrobial properties that act as a preventive agent against caries development. Metal nanoparticles' 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 [33].

The present study showed application of InONPs on demineralized enamel after pH cycling procedure was applied on sound enamel for carious lesion initiation, which was also used by further Iraqi studies [22,31]. 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 vivo situation in which the enamel subjected to de- and remineralizing sequence. The initiation of carious lesion in the current study was conducted in ten days.

After pH cycling, the data from Energy Dispersive Spectroscopy (EDS) study, which demonstrated a decrease in the mean atomic percentage of calcium and phosphorous following demineralization when compared to the sound group, support this (sound teeth). This finding was supported by a SEM micrograph of demineralized enamel surface, which showed a significant number of voids and micro gaps. 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 [34].

Regarding the demineralized group treated with InONPs, The finding of the EDS analysis, showed that there was no increase in the mean atomic percentage of calcium and a reduction in the mean atomic percentage of phosphorous ions as compared to demineralization group, as there was no source for free calcium and phosphorous ions to be re-deposited on the enamel surface and there may be a substitution occurred between phosphorous with indium ions which requires further future studies.

The SEM micrograph confirmed the findings as it revealed that the deposition of the InONPs over the demineralized enamel surface was translucent protective crystalline structures of NPs that occlude the micropores created after demineralization. Since it is unknown exactly what chemical makes up the enamel crystals that would develop following the application of InONPs, the EDS analysis for oxygen showed that the atomic percentages grew and dropped in the opposite way to that of calcium and phosphorous. This was difficult to explain. However, because the elements in the enamel surface were expressed in atomic percentages using the EDS analysis, In order to achieve a balanced composition and a total percentage that does not exceed 100%, an increase in one element's percentage would therefore affect the percentages of other elements in the chemical composition of enamel. This was supported by a prior Iraqi study conducted by Albazaz in 2017 using metal iron oxide nanoparticles for enamel remineralization. Therefore, it is advised that future research make use of cutting-edge technology to expose the crystallographic and chemical alterations that occur following the application of various chemicals to the enamel surface [22].

Using different fluorides can help prevent cavities or demineralization resistance from occurring in the first place. 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 [35], 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 assault is well established from experimental research [36]. Because of this, deionized water was employed as the experiment's negative control and sodium fluoride as the experiment's positive control. On the individual and community levels, it is expensive and technique-sensitive due to the need for various applications. Additionally, worries about toxicity, ingestion, and dental fluorosis have been raised [37]. Due to these disadvantages, it may be beneficial to look for alternatives through scientific research. Another effective anti-caries drug that combines preventative and antibacterial qualities is nano indium oxide.

The hydroxyl group in hydroxyapatite was replaced by a fluoride group, which is fluoride's well-known mode of action (HAP). As a result, the density of HAP crystals increased, making enamel less vulnerable to further acid damage. Remineralization from this method, however, may only be partial in conditions where cavities or severe demineralization were already present because there weren't any undamaged HAP crystal sites. Additionally, different areas of the HAP crystal than fluoride could be coated by metal NPs [37]. Remineralization systems can be further enhanced with more study, opening the door to better and more sophisticated strategies for caries and demineralization prevention.

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