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RESEARCH PAPER

The Impact of ND: YAG Laser and PLGA/ Xylitol Nanoparticles on Dental Enamel (in Vitro Study)

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ABSTRACT

Dental caries is a prevalent chronic infectious illness caused by tooth-adherent cariogenic bacteria. Demineralization by acid invasion is the fundamental principle of a dental caries lesion. One of the possibly effective preventative methods against dental caries is laser and nanotechnology. The objective of this study is to determine the impact of the Nd:YAG laser and PLGA/Xylitol nanoparticles on the microhardness of dental enamel and the morphological change in the dental enamel ultrastructure. In this study, 55 maxillary first premolars were divided into five groups: four study groups and one control group, each with 11 teeth; 10 teeth were examined for microhardness, while one tooth was subjected to SEM. A circular window was positioned on the buccal surface of each tooth. Following a PH cycling technique to activate caries lesions on the tooth enamel, lasing was performed using predetermined parameters. The PLGA/Xylitol nanoparticle concentration was adjusted to 5%. The microhardness and morphological change were measured quantitatively using micro Vickers and SEM, respectively, at 3 stages: sound, demineralization, and treatment. Enamel microhardness values decreased highly significantly after the demineralization stage compared to the sound stage for all groups. After application of various agents, the microhardness values of all treated groups, excluding the control, increased highly significantly in comparison to the demineralization stage. The nanoparticles+laser treated group exhibited a sharp increase in microhardness values. An SEM showed an ultrastructural change that began with the loss of typical enamel structure after demineralization. The application of nanoparticles, nanoparticles + laser, and laser + nanoparticles caused the majority of surface defects to be repaired. The enamel surface treated with Nd:YAG laser and PLGA/Xylitol nanoparticles yielded favorable results in terms of microhardness and SEM analysis, suggesting that this therapy could be recommended as a means of preventing dental caries.

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INTRODUCTION

Enamel is the human body's hardest tissue, and it produces a protective layer of varied thickness across the entire tooth crown. It acts as a semipermeable membrane, allowing partial or complete transport of specific particles [1, 2]. Enamel is composed of the smallest structural units, needle- or plate-like Hydroxyapatite (HAP). HAP crystals, which are calcium phosphate salts, compose the mineral content of dental enamel, resulting in enamel prisms [3, 4]. Dental caries is a multifactorial disorder involving the collaboration

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of bacteria on the teeth's surfaces, dental plaque, and diet, particularly carbohydrate parts of meals, which are fermented to organic acids by the plaque microflora over time [5]. Dental caries are linked to continuous cycles of demineralization and remineralization, with the intervening stages either irreversible or reversible [6]. In spite of dental caries can be prevented, it remains a public health problem, so that priority is prevention of dental caries better than advanced restorative treatment [7]. Lasers have many applications in the medical and dental fields [8]. Neodymium-doped Yttrium Aluminum Garnet (Nd:YAG) is one of the most famous types of laser. It emits light with an infrared wavelength of 1064 nm. Typically, pulsed Nd:YAG lasers are triggered in the Q-switching mode [9]. There are many advantages of laser treatment in comparison to traditional treatment, including decreased hemostasis, tissue swelling, and cellular destruction; reduced post-operative complications; and increased visualization of surgical sites [10].

Nd:YAG laser irradiation was capable of enhancing the acid resistance of dental enamel and capable of damaging pathogenic organisms [11, 12, 13].

Nanotechnology or nanoscience is defined as the investigation and evolution of an applied science at the molecular or atomic level [14]. The particle size is decreased to nanometers, which are between 1 and 100 nanometers this will enable greater presence of atoms on the surface, which provides maximum contact with the environment and makes penetration through cell membranes possible. Hardness, active surface area, chemical reactivity, and biological activity can all be altered, resulting in increased drug release of active therapeutic agents [15, 16, 17, 18]. Nanotechnology has been evaluated in different areas of medical and dental applications including the prevention of dental caries [19, 20, 21,22, 23].

PLGA is a polylactic acid (PLA) and polyglycolic acid (PGA) copolymer. It is the best-characterized biomaterial currently available for drug delivery in terms of performance and design [24]. PLGA nanoparticles may be used in many dental fields, such as dental surgery, endodontic therapy, implantology, cariology, and periodontology [25]. Xylitol is a safe, nonfermentable sugar alcohol that has been considered a cariostatic and noncariogenic agent. When used as gum or mints, they will lead to increased mineral-rich saliva and alkalinity from small salivary glands in the palate. Increased salivary flow leads to higher mineral content and buffering capacity. This will lead to the remineralization of the destructive areas of enamel [26, 27]. Xylitol can activate the remineralization of deeper demineralized enamel layers by easing calcium accessibility and mobility [28]. To increase the activity of xylitol, it can be loaded into PLGA nanoparticles. Xylitol loaded with nanoparticles was successfully improved and led to a reduction in the particle size, an increase in particle surface, and enhanced antibiofilm activity of xylitol [29, 30].

To date, no study has assessed the combination effect of PLGA/ Xylitol. nanoparticles with the Nd:YAG laser on human dental enamel, so this study will be conducted.

MATERIALS AND METHODS

An in vitro study was conducted from August 2022 to November 2022 using 55 maxillary first premolars in Baghdad, Iraq. Ethical approval for the study was conducted at the Department of Pediatric and Preventive Dentistry, College of Dentistry, University of Baghdad, after receiving ethical approval from the University of Baghdad's Ethical Committee (Ref. 560 on April 17, 2022).

Laser irradiation procedure

For irradiating the dental enamel surfaces, an AQ-switched Nd:YAG laser (Model Number: PL755, Wave Length: 1064, 532 nm, China), operating at 532 nm wavelength (according to UV visible measurement in the pilot study). Frequency 1 Hz and powered to 2 nanosecond laser pulses (1 J) (Fig. 1). To achieve the required laser fluence on the enamel surface, a positive lens with a focal length of 10 cm was used. An optical microscope was used to detect a laser spot with a diameter of 0.8 mm by making a burn mark on carbon paper. Lasing was created at the Department of Applied Science at the University of Technology. The out put energy for the laser group is 60 mJ, and the number of pulses is 5.

Preparation of nanoparticles

A solvent evaporation method used for the production of PLGA and xylitol nanoparticles. The concentration of nanoparticles in this experiment was established at 5% based on a pilot study (Fig. 2). In distilled water, xylitol and surfactants were dissolved, while PLGA was dispersed in acetone. Using a sonicator (Bransonic, USA), the organic phase was introduced drop by drop to the aqueous solution, followed by two hours of rotaty evaporation at 40 °C. Using a freeze dryer, the nanoparticles were then frozen at 80 °C for 18 hours and lyophilized at 110 °C for 24 hours (CD-2820, China) [29].

Identification of nanoparticles

Ray Diffraction Pattern (XRD)

It is the technology used to determine the type and phase of a substance without distortion. To detect deviation information, it is necessary for the incident light on the substance to deviate at a specific angle [31]. The X-ray identified the characteristics of nanoparticles by dropping a solution onto glass slides and allowing it to dry. The X-ray radiation source was CuKα (1.54 eV) at an angle of 2θ (10°–80°). This investigation was conducted in the XRD Laboratory of the Nanoscience Department at the University of Technology.

Filed Emission Scanning Electron Microscopy (FESEM)

The FE-SEM is an electron microscope. In a raster-scan fashion, it scans the specimen surface with a high-energy electron beam. Electron emitters from the FE-SEM gun were implemented. It emits up to 1,000 times more light than a tungsten filament. However, they needed higher vacuum levels. After leaving the electron gun, the electron beam is confined and focussed into a thin, monochromatic beam using magnetic lenses and metal apertures. Then, microscopes that collect signals to create an image of the material are equipped with detectors of each type of electron. Images produced by in-lens FESEM are clearer and less electrostatically deformed than those produced by SEM [32].

Fig. 1. ND:YAG laser

Fig. 2. PLGA/ xylitol nanoparticles

Sample preparation

In this study, 55 maxillary first premolars from Iraqi patients aged 12 to 22 seeking orthodontic treatment were collected. Teeth are classified into five types: Four study groups and one control group each had 11 teeth: one tooth for SEM evaluation and ten teeth for microhardnessing.

Group 1: non-treatment with neither lasers nor nanoparticles (deionized water DW).

Group 2: will only be treated with lasers.

Group 3: will be administered PLGA/Xylitol nanoparticles.

Group 4: laser treatment first followed by PLGA/Xylitol nanoparticles (laser + nanoparticles)

Group 5: Will be treated with PLGA/Xylitol nanoparticles followed by laser (nanoparticles+ laser).

A circular window was positioned and standardized on the buccal surface of each tooth. (Fig. 3). This window was scraped and polished to produce a flat surface suitable for SEM and microhardness testing [33]. By preparing demineralizing and remineralizing solutions, PH cycling was used to activate caries lesions on the enamel surface [34].

The microhardness was measured using a digital micro Vickers hardness tester with a 100-gram load for 15 seconds in the Materials Engineering Department Laboratory at the University of

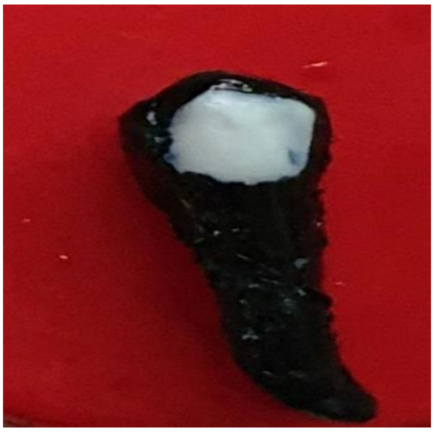
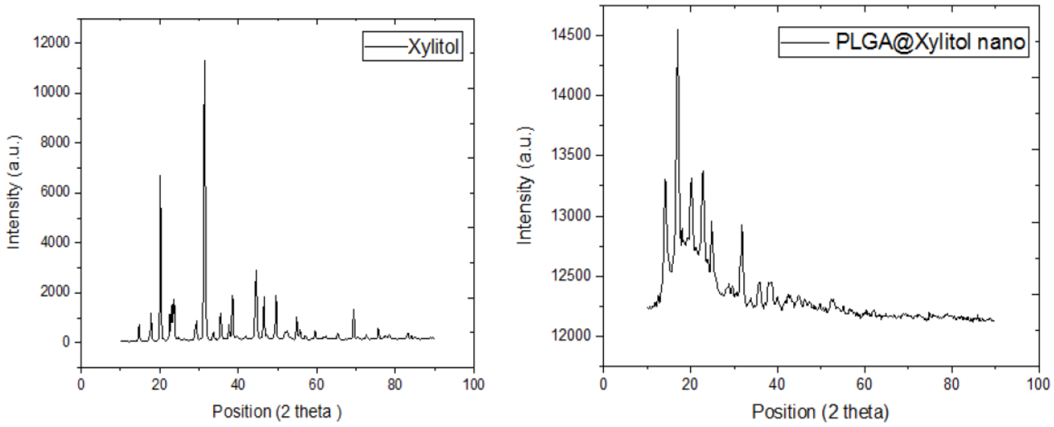


Fig. 3. Preparation of window on buccal surface of maxillary first premolar tooth



A B

Fig. 4. The XRD pattern A- The XRD pattern of xylitol, B- The XRD pattern of PLGA/ Xylitol nanoparticles

Technology. Vickers microhardness measurment was made by optical microscope according to the instruction [35]. Three indentations for each specimen were done , and three identical records were kept. The average of these three records was then determined. A Scanning Electron Microscopy (SEM) examination of one specimen from each group was performed to detect the existence of morphological abnormalities on the enamel surface by scanning the specimen with a focused electron beam [36]. The gold-coated teeth were placed in a machine with a vacuum system to improve the imaging of samples [37, 38].

Statistical Package for Social Research was used for descriptive analysis, and presentation (SPSS version -22, Chicago, Illionis, USA). Levene test for such a quantitative variable that includes the minimum, maximum, mean, standard deviation (SD), and standard error. One way ANOVA test the mean difference for 3 groups and more and using Bonferroni posthoc test, Tukey HSD Two Sided.

Not significant with a P value more than 0.05, significant at a P value lower than 0.05

RESULTS AND DISCUSSION

XRD Analysis

Cu Kα radiation was used to determine the X-ray diffraction results (1.5406 A). Fig. 4 depicts the X-ray diffracted patterns of xylitol and PLGA/xylitol nanoparticles. Indicative of the transformation of the material into nanoparticles, a difference was seen in the breadth of the beak, as xylitol had a high degree of crystallization before it was transformed into nanoparticles, at which point it became less crystallized. The substance’s crystal size was determined using Scherer’s equation.

Filed Emission Scanning Electron Microscopy (FESEM)

The FESEM analysis is a crucial test for determining the morphology of nanoparticles that have been manufactured. The form and size of PLGA/Xylitol nanoparticles were depicted in Fig. 5. The photos depict a heterogeneity of nanoparticles with various sizes and shapes. The particle sizes are smaller than 100 nm.

Microhardness values (mean and standard deviation) of enamel surfaces treated with different agents

The mean microhardness values for the sound, demineralization, and treatment stages were determined. The ANOVA test revealed that there was no significant difference in the microhardness values between the groups for either sound or demineralization (p>0.05).

Table 1. Normal distribution among groups using Shapiro Wilk test at p>0.05.

Groups	Shapiro-Wilk								
	Base line			Demineralization			Treatment		
	Statistic	df	P value	Statistic	df	P value	Statistic	df	P value
Control DW	0.849	10	0.057	0.853	10	0.062	0.928	10	0.426
ND:YAG	0.858	10	0.072	0.869	10	0.098	0.875	10	0.114
Nano	0.895	10	0.191	0.880	10	0.132	0.942	10	0.575
ND:YAG+Nano	0.933	10	0.476	0.944	10	0.596	0.909	10	0.276
Nano+ND:YAG	0.855	10	0.066	0.859	10	0.074	0.916	10	0.325

DW: Deionized water group
Nano: PLGA/Xylitol nanoparticles group

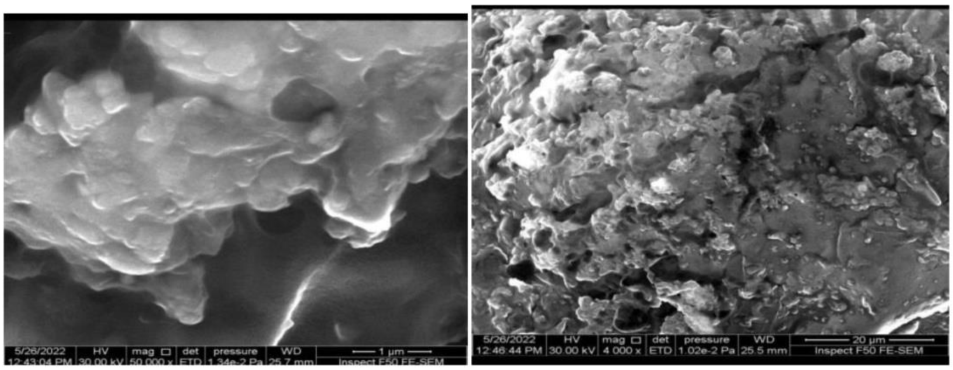


Fig. 5. FESEM of PLGA/ Xylitol nanoparticles

During the remineralization stage, statistically highly significant variations between groups were observed ($p < 0.001$) (Table 2). Following the demineralization stage, all groups experienced a highly significant decline in microhardness values relative to the microhardness values of the sound teeth stage. The application of different treatments (remineralization stage) resulted in a highly significant increase in microhardness values for all treated groups except the control group compared to the demineralization stage (Table 3).

During the remineralization stage, when the microhardness values of all groups are compared, statistically highly significant differences between the control group and all treatment groups are found ($p<0.001$). When ND:YAG group was compared to the nanoparticles + ND:YAG groups, there was a significant difference ($p < 0.05$). By comparing the groups treated with nanoparticles and nanoparticles + ND:YAG , a statistically significant difference was shown. There were no significant differences between the other groups ($p > 0.05$). In comparison to other treatments, the control (de-ionized water) has a minor change in microhardness. Treatment with nanoparticles +lasers lead to the greatest change in microhardness among the other groups, while treatment with lasers resulted in the least change

Table 2. Descriptive and statistical test of surface microhardness among groups and phases.

		DW	ND:YAG	Nano	ND:YAG+Nano	Nano+ND:YAG	F	p
Baseline	Minimum	295.500	251.330	286.630	280.000	252.800	1.608	0.189 NS
	Maximum	370.130	413.900	350.500	338.000	390.900		
	Mean	326.113	323.249	332.683	305.409	347.704		
	±SD	25.616	55.019	24.694	17.246	52.255		
Demineralization	Minimum	150.500	161.930	158.030	146.370	189.870	2.314	0.052 NS
	Maximum	208.000	212.530	210.770	207.270	235.000		
	Mean	188.867	194.176	179.530	189.827	213.134		
	±SD	20.805	19.056	19.061	17.677	18.519		
Treatment	Minimum	155.000	251.210	211.980	258.330	292.370	23.010	0.000 Sig.
	Maximum	302.150	298.170	283.630	281.930	310.200		
	Mean	202.778	269.920	258.264	274.685	301.429		
	±SD	39.989	19.402	28.421	6.323	7.398		
F		48.644	63.155	78.175	67.030	78.192		
p		0.000	0.000	0.000	0.000	0.000		
Effect size		0.689	0.742	0.7803	0.753	0.7804		
MHR	Minimum	-7.582	40.060	33.051	65.336	43.342		
	Maximum	61.043	156.027	69.788	94.962	183.981		
	Mean	8.954	73.562	50.227	74.441	81.757		
	±SD	19.664	47.075	12.989	10.968	54.625		

DW: Deionized water group
Nano: PLGA/Xylitol nanoparticles group

Table 3. Multiple pairwise comparisons of surface microhardness among phases by groups using Bonferroni posthoc test.

Groups	Phases		Mean difference	p value
DW	Baseline	Demineralization	137.246	0.000
		Treatment	123.335	0.000
	Demineralization	Treatment	-13.911	0.250
ND:YAG	Baseline	Demineralization	129.073	0.000
		Treatment	53.329	0.001
	Demineralization	Treatment	-75.744	0.000
Nano	Baseline	Demineralization	153.153	0.000
		Treatment	74.419	0.000
	Demineralization	Treatment	-78.734	0.000
ND:YAG+Nano	Baseline	Demineralization	115.582	0.000
		Treatment	30.724	0.076
	Demineralization	Treatment	-84.858	0.000
Nano+ND:YAG	Baseline	Demineralization	134.570	0.000
		Treatment	46.275	0.003
	Demineralization	Treatment	-88.295	0.000

DW: Deionized water group
Nano: PLGA/Xylitol nanoparticles group

in comparison to the demineralization (Table 4).

Microscopic features of the outer enamel surface using Scanning Electron Microscope (SEM)

The structural alterations of the enamel surface for each group are depicted in the figures below.

Fig. 6 depicts the typical, undamaged, and smooth enamel surface structure of the control group (sound enamel), with normal perikymata arranged in parallel lines with few holes. The demineralization group's enamel surface structure has changed. The prisms exhibited irregularity, causing the enamel to deviate from its normal construction. As seen in Fig. 7, there are numerous micropores and cavities on the enamel surface.

Fig. 8 shows a SEM image of an enamel surface treated with a Nd:YAG laser. Examination revealed the disappearance of normal enamel perikymata, the formation of microgaps, craters, fissures, and unique melted and recrystallized areas. SEM picture of an enamel surface treated with nanoparticles (Fig. 9) the existence of globular, crystalline, and amorphous structures that occlude the micropores created during the demineralization stage.

Fig. 10 shows the SEM of the group (laser + nanoparticles); the deformation caused by laser radiation was corrected by nanoparticle precipitation. Fig. 11 depicts the structural alteration caused by the group (nanoparticles + laser), with the majority of the micropores occluded and hidden. Deposition of nanoparticles scattered on the enamel surface and filling surface defects reduced the surface roughness caused by

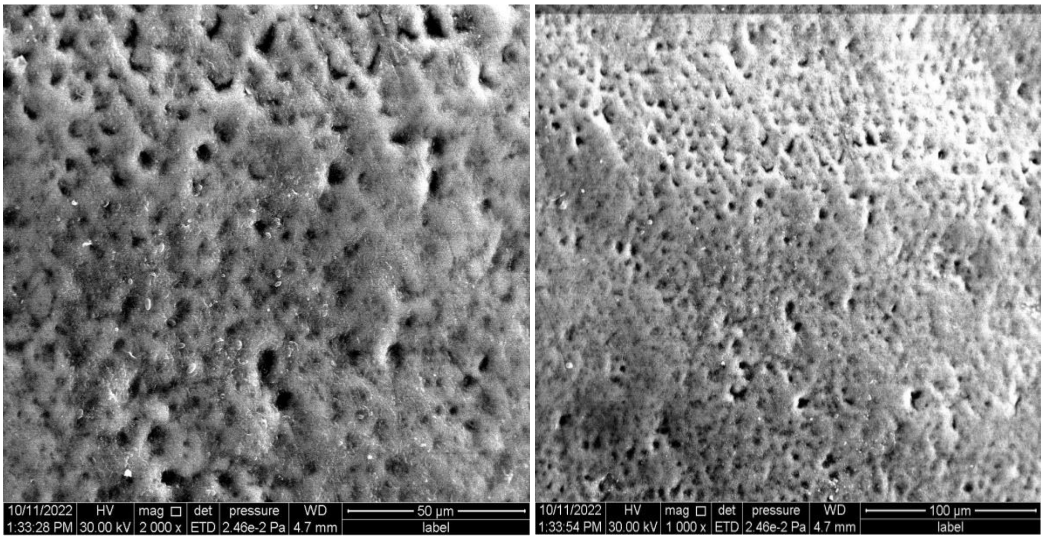


Fig. 6. SEM for normal sound enamel surface

Table 4. Multiple pairwise comparisons of surface microhardness among groups by phases using (Tukey HSD)

Groups		Mean difference	Tukey HSD p value
DW	ND:YAG	-67.142	0.000
	Nano	-55.486	0.000
	ND:YAG+Nano	-71.907	0.000
	Nano+ND:YAG	-98.651	0.000
ND:YAG	Nano	11.656	0.813
	ND:YAG+Nano	-4.765	0.992
	Nano+ND:YAG	-31.509	0.040
Nano	ND:YAG+Nano	-16.421	0.549
	Nano+ND:YAG	-43.165	0.002
ND:YAG+Nano	Nano+ND:YAG	-26.744	0.110

DW: Deionized water group
Nano: PLGA/Xylitol nanoparticles group

laser radiation. Within the pores, nanoparticles create a plug.

Dental caries is still a widespread public health issue. Despite the fact that there are numerous strategies to avoid it, there is still a need to develop new strategies for implementing comprehensive preventive programs. Laser and nanotechnology are two of the potentially effective preventive measures.

A pH cycling technique was developed to activate carious lesions [39]. This method is viewed as a better encouragement of the in vivo condition in which the enamel is subjected to a de- and remineralizing sequence at the onset of dental caries. In the current investigation, this process took ten days [39, 40, 41]. All groups had their enamel microhardness measured at 3 stages (sound, demineralization, and treatment). At demineralization and the beginning of a dental caries lesion, there is a statistically highly significant decrease in the microhardness of the enamel surface in comparison to the sound tooth surface. This is due to the fact that any drop in the pH of the surrounding environment under the critical pH (5.5) will create an acidic environment, causing the tooth minerals, phosphorous and

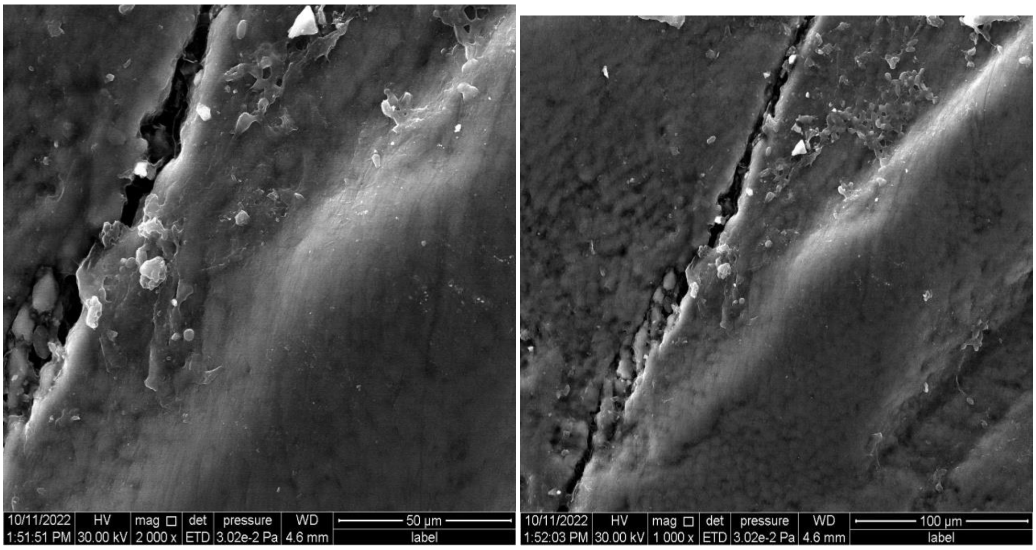


Fig. 7. SEM for demineralized enamel surface

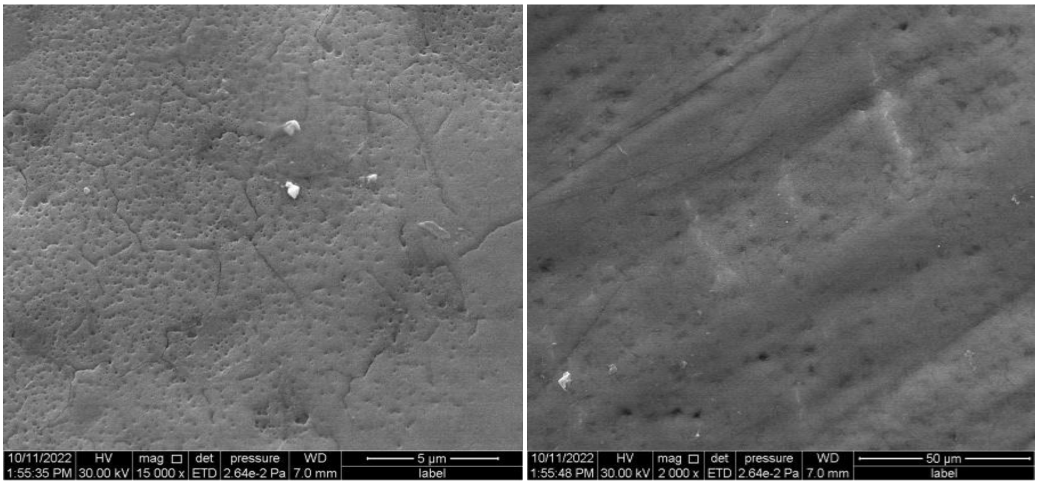


Fig. 8. SEM for enamel surface treated with laser

calcium in particular, to move outward, leaving micropores and decreasing microhardness [42]. This result was supported by SEM micrograph of the demineralized stage that showed many microspaces and voids. For the all-treated group, the results showed that microhardness was rising with statistically highly significant differences in comparison to the demineralization stage.

Studies showed that laser irradiation causes a rise in temperature in dental tissues. The chemical composition and structure of dental enamel are altered when the temperature rises [43,44]. These changes include heat recrystallization, the loss of water, a decrease in carbonate, and an increase in crystal size, as well as the creation of tricalcium phosphate and pyrophosphate through the condensation of acid phosphate ions [45]. Pyrophosphate inhibits the disintegration of crystals, but tri- and tetracalcium phosphates are more acid-sensitive than hydroxyapatite [46, 47].

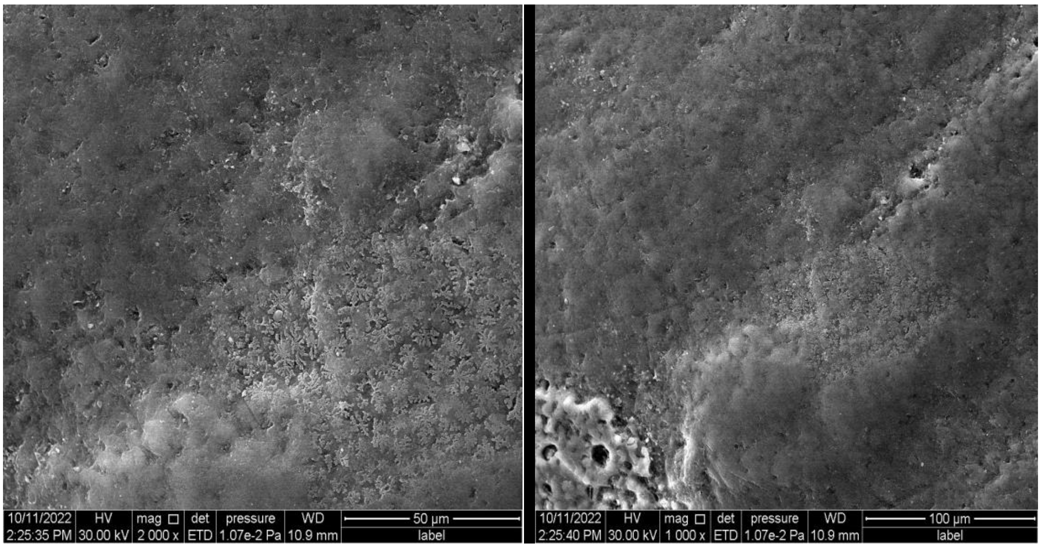


Fig. 9. SEM for enamel surface treated with nanoparticles

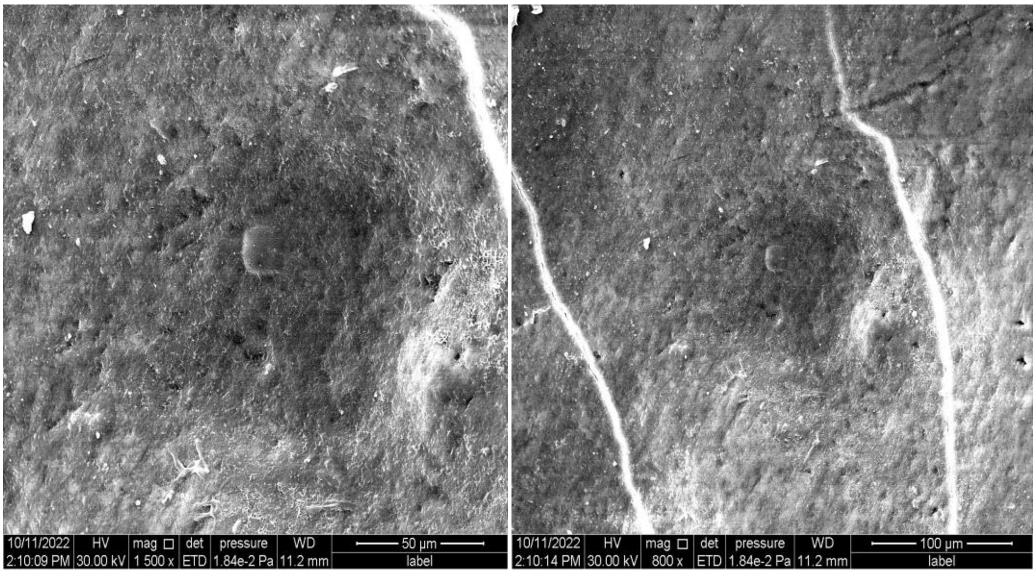


Fig. 10. SEM for enamel surface treated with ND:YAG+Nanoparticles

Enamel becomes more resistant to acid attack [11, 12]. This may also be attributed to evidence that laser wave length is consistent with the absorption peak of hydroxyapatite crystal, which is the main constituent of dental enamel, and then greatly absorbed and effectively transformed to heat without destroying the surrounding or underlying tissues, resulting in modifications of the ultrastructural enamel surface (melting and re-crystallization) and increased microhardness [48].

The current laser study's findings agree with those of A. Alkaisi and B. Salma, 2021, who established that the Nd:YAG laser is effective for improving the microhardness of the dental enamel with the least morphological defect by employing low energy with more pulses [47]. The findings disagree with M. Majori, 2005, who found no significant differences between treated and untreated tooth enamel samples [49]. This was most likely owing to the several variables involved in the lasing process, such as power, pulse frequency, and irradiation time.

Melting and re-solidification processes will be demonstrated by the formation of microspaces (holes) as a consequence of water evaporation from the tooth enamel matrix, as demonstrated by a SEM micrograph.

Xylitol, also known as $(CHOH)_3(CH_2OH)_2$, is a safe sugar alcohol sweetener that does not cause caries by preventing bacterial fermentation [50]. Xylitol can promote phosphate and calcium ion transit for the remineralization of demineralized enamel, which can concentrate calcium [28, 51, 52]. Study showed that PLGA loaded with the material increased the microhardness [53]. There is no previous study to compare the effect of PLGA/ Xylitol nanoparticles on tooth enamel, so compare with studies including xylitol. The findings of this study support the findings of prior studies that found xylitol to have the highest remineralizing property [54, 55]. The SEM micrograph showed amorphous, crystalline, and globular formations that formed on the surface of the enamel and filled the microgaps.

When compared to the demineralization stage, there was a statistically highly significant difference in the mean values of microhardness for the group treated with laser followed by nanoparticles. The most likely explanation is that laser treatment first creates microgaps (holes) that promote ion incorporation, and then these microgaps are closed with nanoparticles [56]. The SEM micrograph revealed that the precipitation of a nanoparticle layer corrected the majority of the surface crakes and flaws caused by laser. The mean values of microhardness increased with a statistically highly significant difference in the group treated with nanoparticles followed by laser irradiation, which may be due to the synergistic effect of nanoparticle incorporation in the outer enamel structure with the process

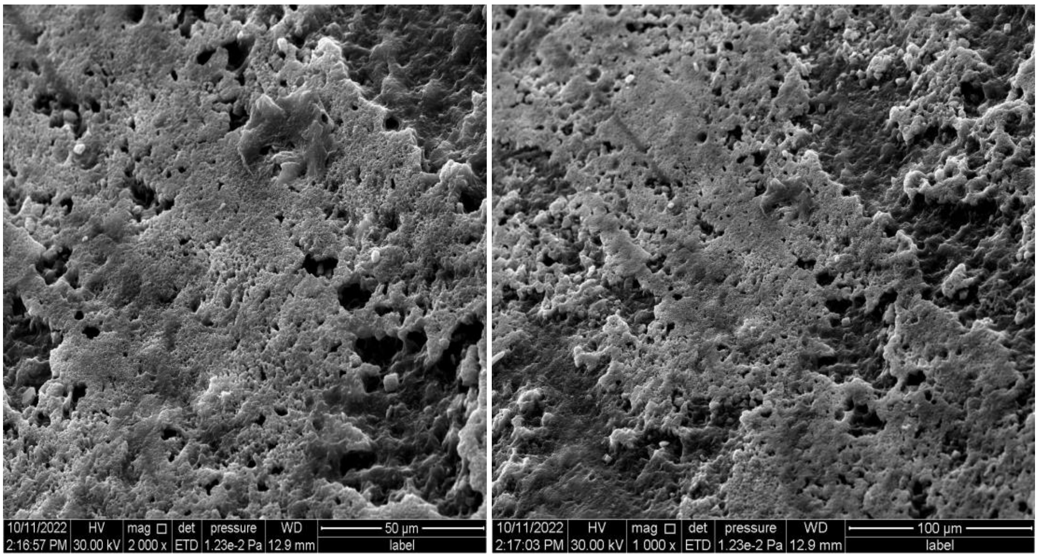


Fig. 11. SEM for enamel surface treated Nano particles +ND:YAG

of melting and re-solidification caused by laser irradiation. Another hypothesis is that when the enamel surface was exposed to nanoparticles followed by laser, the laser assisted in the ion connecting of the remineralizing agent [57, 58, 59, 60]. This is also confirmed in the current work by SEM micrographs, which demonstrated that the majority of the microspaces were closed by nanoparticles that were recrystallized, melted, and trapped in the microspaces, forming a plug.

There was no prior research with which to compare the outcomes of this experiment. When compared to the control group, there is a statistically highly significant difference in microhardness values for all groups. The nanoparticles + laser group produced the most remineralization as the highest number was reported compared to the demineralization stage. Laser + nanoparticles came next, then the nanoparticles group, then the laser group, coming just before the control (teeth treated with de-ionized water), which recorded the least changes. This could be owing to the synergistic action of the combined technique, which causes values to grow more than microhardness values when laser and nanoparticles are employed separately.

The highest rise in the group of nanoparticles followed by lasers, as this conclusion may roughly match the finding of the study by S. Valizadeh et al., 2020. It was demonstrated that applying a treatment agent prior to irradiation is superior to irradiation followed by the application of a treatment agent [61]. Which may be due to the synergistic effect of nanoparticle incorporation in the outer enamel structure with the process of melting and re-solidification caused by laser irradiation [57-60]. Although all of the agents tested raised the mean values of the microhardness of dental enamel, none of them produced results that were close to the original microhardness values of sound teeth. This could be due to the current study's short application time. Increased application time to weeks rather than one week may raise microhardness values; nevertheless, additional research will be required in the future to evaluate whether the hardness approaches the original value or is enhanced higher.

CONCLUSION

The treatment of the tooth enamel surface with nanoparticles and laser produced good results in terms of microhardness and SEM evaluation, which might be attributed to the synergistic action of the laser and nanoparticles. This treatment could be considered for the prevention of dental cavities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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