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The Impact of Cold Atmospheric plasma and PLGA/ Xylitol Nanoparticles on Dental Enamel (in Vitro Study)

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Abstract

Plasma and nanotechnology are potentially effective preventive measures against dental caries. This study aims to determine the impact of cold atmospheric plasma and PLGA/Xylitol nanoparticles on dental enamel microhardness and morphological changes in dental enamel ultrastructure. In this study, 56 maxillary first premolars were divided into five groups: one control group, and four study groups, each with 11 teeth; 10 teeth were examined for microhardness; and one tooth was examined using FESEM. A circular window was placed on the buccal surface of each tooth. Following a PH cycling technique to activate caries lesions on the tooth enamel. cold plasma was performed using predetermined parameters. The PLGA/Xylitol nanoparticle concentration was adjusted to 5%. The microhardness and morphological change were measured using micro-Vickers and FESEM respectively, at three stages: sound, demineralization, and treatment. Enamel microhardness values decreased highly significantly after the demineralization stage compared to the sound stage for all groups. After treatment, the microhardness values of all treated groups, excluding the control, increased highly significantly in comparison to the demineralization stage. The group nanoparticles + plasma showed the highest microhardness recovery (82.559± 23.596), followed by plasma + nanoparticles (74.774±18.302), while samples treated with nanoparticles only showed the lowest recovery in the microhardness among all study groups (50.227±12.989). A FESEM showed that the application of nanoparticles, nanoparticles + plasma, and plasma + nanoparticles caused many surface defects to be repaired. The enamel surface treated with cold atmospheric plasma and PLGA/Xylitol nanoparticles yielded favorable results in terms of microhardness and FESEM analysis, suggesting that this therapy could be recommended as a means of preventing dental caries.

Keyword: Cold atmospheric plasma, PLGA/ Xylitol Nanoparticles, dental enamel

1. Introduction

The enamel is the exposed tooth's outer layer. It is a strong, thin, translucent coating of calcified substance that envelops and protects dentin [1]. The most mineralized tissue in the human body is tooth enamel. The distinctive mechanical properties of tooth enamel are determined by the different forms and structures of enamel crystals, which include increased microhardness and

resistance to fracture and acid degradation [2]. Dental caries may develop because of the continual remineralization (mineral gain) and demineralization process [3]. When the rate of demineralization overcomes the rate of remineralization, calcium and phosphate ions diffuse out of the enamel, resulting in a chalky white spot lesion that, if not controlled, can develop into cavitation [4]. Pathological variables shift the balance in the direction of dental caries and disease progression, whereas protective factors include salivary components, fluoride together with calcium and phosphate enhance remineralization of dental caries lesions [5].

Plasma and nanotechnology are potentially effective preventive measures against dental caries [6,7]. Cold atmospheric plasma [CAP] is currently being used in medicine. Plasma is an ionized gas with an almost neutral charge. It is often called the "fourth state of matter". The non-thermal atmospheric pressure doesn't go above 50°C, so human cells can tolerate it [8]. CAP jets are a kind of cold plasma discharge that generates a high-velocity stream of highly reactive chemical species and weak emitted light [9]. There are many applications of CAP in dentistry and in cariology [10-13]. Plasma surface treatment has been proposed as a strategy for assisting mineral re-crystallization. Following cold plasma therapy of demineralized enamel, significant increases in mineral volume recovery and microhardness of the demineralized region were found [7, 14].

Nanotechnology or nanoscience is defined as a technology is associated with small materials or structures that are smaller than 100 nm in at least one dimension [15]. The particle size is decreased to nanometers which provides maximum contact with the environment and makes penetration through cell membranes possible. hardness, mechanical properties, chemical reactivity, and biological activity can all be altered, resulting in increased drug release of active therapeutic agents [16-18]. Nanotechnology has been evaluated in different areas of medical and dental applications including the prevention of dental caries [19-24]. PLGA is a polylactic acid (PLA) and polyglycolic acid (PGA) copolymer. It is the best-characterised biomaterial currently available for drug delivery in terms of performance and design [25]. PLGA nanoparticles may be used in many dental fields [26]. Xylitol is a tooth-friendly, nonfermentable sugar alcohol that has been considered a cariostatic and noncariogenic agent. [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 led to a reduction in particle size, an increase in particle surface, and enhanced antibiofilm activity of xylitol [29, 30]. Surface microhardness (SMH) evaluation is a simple, quick and easy to measure, non-destructive method, reflecting mineral changes that have occurred due to the therapeutic procedures [31].

2. Materials and Methods

An in vitro study was conducted from August 2022 to November 2022 using 56 maxillary first premolars in Baghdad, Iraq. Ethical approval for the study was the from Ethical Committee of the University of Baghdad, College of Dentistry (Ref. 560 on April 17, 2022).

2.1. Plasma Procedure

The CAP groups' samples were treated with an Iraq-made cold atmospheric plasma jet employing argon gas at a flow rate of 10 L/min, 175 volts, and a frequency of 2.45 GHz at room temperature. The maximum distance between the nozzle tip and the enamel surface was 2 mm. The time for plasma therapy was set at one minute **Figure 1**.

Figure 1. Cold atmospheric plasma jet

2.2.Preparation of nanoparticles

Solvent evaporation method used to produce PLGA/ xylitol nanoparticles. The concentration of nanoparticles in this experiment was established at 5%. (**Figure 2**). In distilled water, xylitol and tween (surfactant) were dissolved, while PLGA was dispersed in acetone. Using a sonicator, the organic phase was introduced drop by drop to the aqueous solution, followed by two hours of rotary evaporation at 40 °C. Using a freeze dryer, the nanoparticles were frozen at 80 °C for 18 hours and lyophilized at 110 °C for 24 hours [29]. The treatment time is 4 minutes daily for 7 days.

Figure 2. PLGA/ xylitol nanoparticles

2.3. Identification of PLGA/ Xylitol Nanoparticles

2.3.1. Ray Diffraction Pattern (XRD)

It is one of the most often used measurement techniques for identifying a substance's nature and phase without causing any damage. To expose the diffraction information, it depends on the incident ray's diffraction on the material to scatter at a specific angle [32]. When the suspension of the produced nanoparticles was deposited onto glass slides and allowed to dry, an X-ray was used to characterize them. Cu-Kα radiation at a wavelength (λ = 0.15406 nm) was used as the X-ray radiation source at a 2° angle (10°-80°).

2.3.2. Filed Emission Scanning Electron Microscopy (FESEM)

FE-SEM is a specific kind of electron microscope that generates an image by moving a high-energy electron beam across the sample surface in a raster scan pattern. The pictures produced by the FESEM lens are clearer and less electrostatically deformed than those produced by SEM [33].

2.3.3. Sample Preparation

Teeth are classified into five groups: one control group, and four study groups, and each tooth had 11: one tooth for SEM evaluation and ten teeth for microhardness.

Group 1: Control (Deionized water), Group 2: will only be treated with plasma.

Group 3: will be treated with PLGA /Xylitol nanoparticles. Group 4: will be treated with plasma then PLGA/Xylitol nanoparticles (plasma + nanoparticles)

Group 5: will be treated with PLGA/Xylitol nanoparticles and then plasma (nanoparticles+ plasma).

Each tooth's buccal surface, a circular opening was positioned and standardized. This window was polished and ground to generate a flat surface suitable for FESEM and microhardness testing [34]. Using demineralizing and remineralizing solutions, PH cycling was utilized to activate enamel surface caries lesions in the current study, carious lesion initiation occurred within ten days [35]. Microhardness was measured using a digital micro-Vickers hardness instrument with a 100-gram load for 15 seconds. The Vickers hardness was measured using an optical microscope. Each specimen received three indentations. Then, the average of these three records was calculated. The extent of remineralization was calculated as the percent of surface microhardness recovery (%SMHR), and was measured according to the following formula [36]:

$$\%\mathrm{SHR} = \frac{(\mathrm{SH2} - \mathrm{SH1})}{(\mathrm{SH0} - \mathrm{SH1})} \times 100 \quad (1)$$

Where SH0 was the baseline surface hardness, SH1 was the demineralization surface hardness and SH2 was the remineralization surface hardness. By scanning the specimen with a focused electron beam, morphological abnormalities on the enamel surface were detected using FESEM.

3. Result

3.1. Identification of Nanoparticles

3.1.1. XRD Analysis

The XRD pattern of PLGA/ Xylitol nanoparticles compared with xylitol is in **Figure 3**. The XRD pattern for xylitol shows a perfect matching with ICDD 34-1802 [37]. The matching occurs at (2θ= 13.97,14.50,17.54,19.82, 22.56, 24.64, 28.13, 29.28, 30.16, 35.36 and 38.26). The XRD test for PLGA/ Xylitol nanoparticles shows an increase in intensity in comparison to xylitol. The substance's crystal size was determined using Scherer's equation [38]:

$$D = \frac{K\lambda}{\beta COS\theta} \quad (2)$$

Where D is crystal size**,** λ is the x-ray wavelength, β is the full width at half maximum of the XRD peak, and θ is the Bragg angle. The average crystalline size of PLGA/ Xylitol nanoparticles is 8.20 nm.

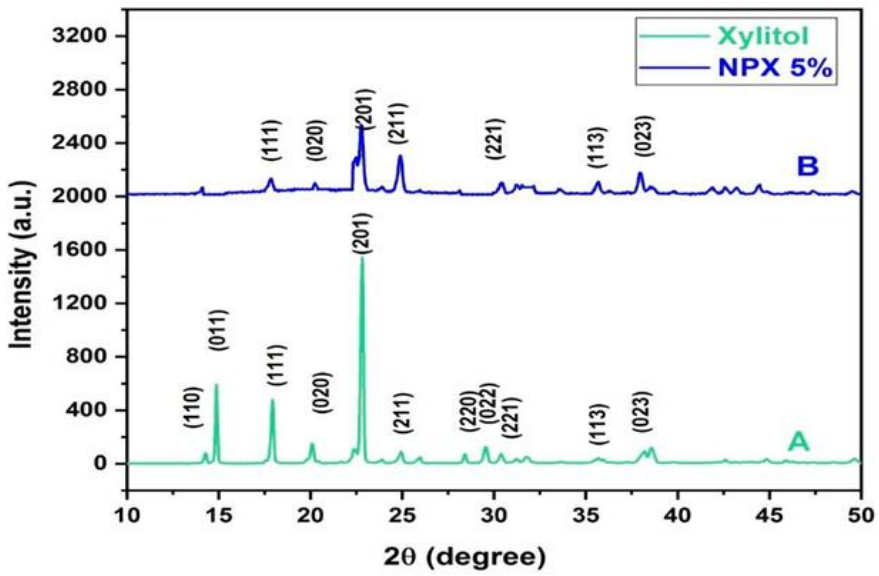

Figure 3. The XRD pattern of PLGA/ Xylitol nanoparticles in comparison with xylitol. NPX 5% = PLGA/Xylitol nanoparticles 5%

3.1.2. 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 are depicted in Figure 4. The photos depict a heterogeneity of nanoparticles with various shapes.

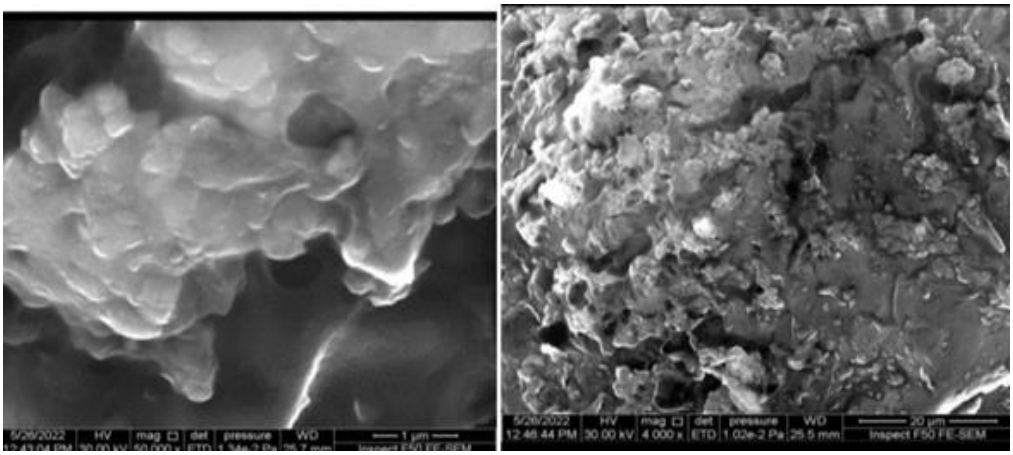

Figure 4. FESEM of PLGA/ Xylitol nanoparticles

3.1.3. Microhardness Value 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 ($p > 0.05$). During the remineralization stage, statistically highly significant variations between groups were observed ($p < 0.001$). The group nanoparticles + plasma showed the highest microhardness recovery (MHR), followed by plasma+ nanoparticles, while samples treated with nanoparticles only showed the lowest recovery in the microhardness among all other groups in **Table 1**.

Table 2 shows the mean difference among the three stages within the same group. Following the demineralization stage, all groups experienced a highly significant decline in microhardness values

relative to the sound teeth stage. In comparison to the demineralization stage, the administration of different treatments (remineralization stage) resulted in a highly significant rise in microhardness values for all treated groups except the control group. The group treated with nanoparticles + plasma had the greatest mean difference in microhardness between demineralization and remineralization stage values, followed by plasma + nanoparticles. Table 3 compares microhardness values for all groups during the remineralization stage. There are statistically significant differences ($p < 0.05$) when the microhardness values of the control group are compared to those of all study groups. There are significant variations between the plasma group and nanoparticles + plasma groups, as well as between the nanoparticles and the nanoparticles + plasma groups. No significant differences were shown among other groups ($p > 0.05$).

3.1.4. Microscopic Features of the Outer Enamel Surface Using FESEM

The structural alterations to the enamel surface for each group are depicted in **Figure 5**. 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 irregularities, causing the enamel to deviate from its normal construction, there are numerous micropores and cavities on the enamel surface. The image of an enamel surface treated with plasma revealed the formation of craters, cavities, and gaps. FESEM picture of an enamel surface treated with nanoparticles showed the existence of globular, crystalline, and amorphous structures that occlude the micropores created during the demineralization stage of the FESEM of the group (plasma and nanoparticles); the deformation caused by plasma radiation was corrected by nanoparticle precipitation. The group (nanoparticles plasma) indicated that nanoparticles created more homogeneity, and the denser mineral content obliterated the plasma-formed surface pores.

Table 1. Descriptive and statistical test of surface microhardness (HV unit) among groups and phases.

		DW	Plasma	Nano	Plasma+Nar	Nano+Plasn	F	p
Baseline	Minimun	295.500	274.100	286.630	293.970	271.400	1.429	0.240 NS
	Maximun	370.130	346.270	350.500	388.870	375.000		
	Mean	326.113	305.474	332.683	330.215	332.873		
	±SD	25.616	27.758	24.694	34.421	37.567		
Demineralizatio	Minimun	150.500	116.500	158.030	179.530	170.500	0.867	0.491 NS
	Maximun	208.000	257.170	210.770	214.430	212.000		
	Mean	188.867	188.229	179.530	197.212	198.473		
	±SD	20.805	47.122	19.061	12.284	15.675		
Treatment	Minimun	155.000	198.400	211.980	274.000	290.000	18.282	**0.000 Sig.**
	Maximun	302.150	295.000	283.630	312.870	320.000		
	Mean	202.778	264.048	258.264	290.118	302.324		
	±SD	39.989	37.625	28.421	10.341	11.202		
F		85.059	56.358	81.972	78.896	92.046		
p		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**		
Effect size		0.795	0.719	0.788	0.782	0.807		
MHR	Minimun	-7.582	50.369	33.051	45.247	65.448		
	Maximun	61.043	82.524	69.788	96.605	132.244		
	Mean	8.954	66.866	50.227	74.774	82.559		
	±SD	19.664	12.873	12.989	18.302	23.596		

Table 2. Multiple pairwise comparisons of surface microhardness among phases by groups using the Bonferroni postdoc 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
Plasma	Baseline	Demineralization	117.245	**0.000**
		Treatment	41.426	**0.000**
	Demineralization	Treatment	-75.819	**0.000**
Nano	Baseline	Demineralization	153.153	**0.000**
		Treatment	74.419	**0.000**
	Demineralization	Treatment	-78.734	**0.000**
Plasma+Nano	Baseline	Demineralization	133.003	**0.000**
		Treatment	40.097	**0.000**
	Demineralization	Treatment	-92.906	**0.000**
Nano+Plasma	Baseline	Demineralization	134.400	**0.000**
		Treatment	30.549	**0.007**
	Demineralization	Treatment	-103.851	**0.000**

Table 3. Multiple pairwise comparisons of surface microhardness among groups by phases using Tukey's HSd (Honestly significant difference)

Groups		Mean difference	Tukey HSD p value
DW	Plasma	-61.270	**0.000**
	Nano	-55.486	**0.001**
	Plasma+Nano	-87.340	**0.000**
	Nano+Plasma	-99.546	**0.000**
Plasma	Nano	5.784	0.991
	Plasma+Nano	-26.070	0.261
	Nano+Plasma	-38.276	**0.033**
Nano	Plasma+Nano	-31.854	0.108
	Nano+Plasma	-44.060	**0.010**
Plasma+Nano	Nano+Plasma	-12.206	0.872

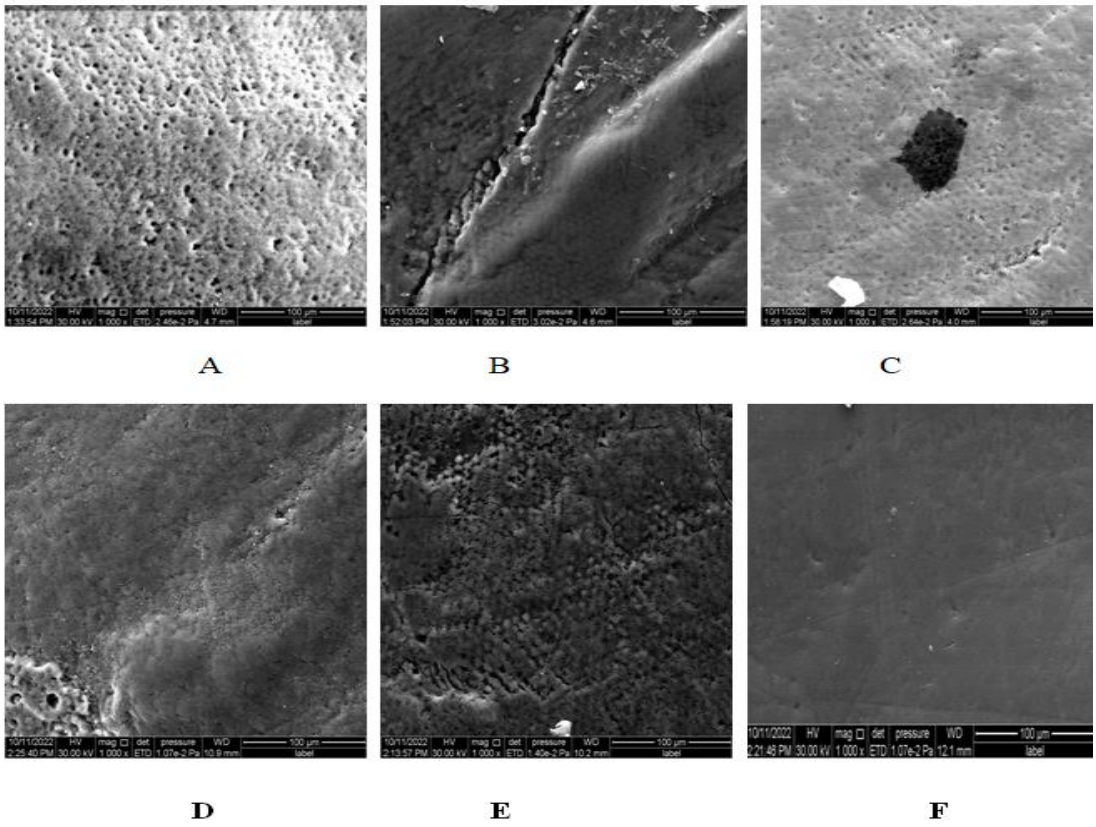

Figure 5. A- FESEM for normal sound enamel surface. B- FESEM for demineralized enamel surface. C- FESEM for enamel surface treated with plasma D-FESEM for enamel surface treated with nanoparticles. E-: FESEM for enamel surface treated with plasma nanoparticles. F- FESEM for enamel surface treated with nanoparticle + plasma

4. Discussion

The XRD test showed that PLGA/ Xylitol nanoparticles matched xylitol in the 2θ position**.** The FESEM showed a heterogeneity of nanoparticles with various shapes. Enamel microhardness was tested for all groups (sound, demineralization, treatment with selected agents). When compared to the sound tooth surface, there is a statistically highly significant decrease in the microhardness of the enamel surface during demineralization and the addition of a dental caries lesion. This is because any drop in the pH of the surrounding environment under the critical pH (5.5) creates an acidic environment, leading the tooth minerals, to move outward, creating micropores and lowering microhardness [39]. This finding was verified by an SEM micrograph of the demineralized stage, which revealed numerous 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.

For the plasma group alone, the microhardness increased due to cold plasma treatment can improve enamel surface characteristics by improving surface energy, wettability, and hydrophilicity of substrates without modifying the bulk structure or increasing pulp temperature [7]. This finding coincides with Šantak et al, 2017, who showed that cold atmospheric plasma jet (APPJ) treatment is a promising technique for chemical surface modification of hard human tooth tissues [40]. The findings disagree with Khoubrouypak et al., in (2021), who found that CAP application had no significant impact on enamel erosion resistance [41]. This may be due to the different variables used. The FESEM image of an enamel surface treated with plasma revealed the formation of craters, cavities, and microspaces (holes), which may be due to melting and re-solidification processes. The mean values of microhardness increased for the group treated with PLGA/Xylitol

nanoparticles. The results of the current study go with the results of other studies that found xylitol to have high remineralizing properties [42,43]. The possible explanation is that xylitol can encourage remineralization of deeper demineralized enamel layers by promoting Ca+2 movement and accessibility and forming complexes with calcium ions and phosphate ions, preventing more general calcium [44]. Another reason may be that PLGA loaded with the particles displayed an increase in microhardness [45].

For the group treated with plasma+ nanoparticles, the mean values of microhardness increased, this may be because cold plasmas consider surface pretreatment, which increases the surface hydrophilicity, wettability, and surface energy of tooth surfaces, which leads to close contact of materials with teeth and is vital to promoting material interaction [7, 46]. Another explanation is that the synergistic effect of cold plasma and nanoparticles has been proposed to improve mineral recrystallization [47]. A FESEM micrograph confirmed this result by revealing that the precipitation of a nanoparticle layer corrected most of the surface cracks and flaws caused by plasma. For the group treated with nanoparticles+ cold plasma. The mean values of microhardness increased, this may be because cold plasmas may increase treatment adhesion to enamel and cause more treatment absorption [48]. This is also confirmed in the current study by FESEM micrographs, which revealed that most of the microspaces were closed by nanoparticles, which formed a more homogeneous and denser mineral content, obliterating the surface porosities formed by plasma. When the control group was compared to the other study groups, there were statistically significant variations in microhardness values; this may be due to the efficacy of all treatments used. When comparing treatments, there is a significant difference between the plasma group and the nanoparticles + plasma group, as well as between the nanoparticles group and the nanoparticles + plasma group. There were no significant differences shown among other groups ($p > 0.05$).

The changes in the microhardness recovery values after treatment with different agents were measured by certain equations, The group nanoparticles + plasma showed the highest microhardness recovery (82.559±23.596), followed by plasma + nanoparticles (74.774±18.302), while samples treated with nanoparticles only showed the lowest recovery in the microhardness among all other groups (50.227 ±12.989). This could be due to the synergistic effect of both agents, which causes an increase in values more than the microhardness values when the technique is used alone. This result couldn’t be compared with other studies' results as there was no previous study that used such a combination of agents. A FESEM showed that the application of nanoparticles, nanoparticles + plasma, and plasma + nanoparticles caused most of the surface defects to be repaired. Nanoparticles + plasma groups produced a better image of FESEM than another group because produced more homogeneous and denser mineral content.

5. Conclusion

The treatment of the tooth enamel surface with PLGA/Xylitol nanoparticles and plasma produced good results in terms of microhardness and FESEM evaluation. PLGA/Xylitol nanoparticle + cold plasma group produced maximum remineralization ability which is assessed by high microhardness recovery and better image of FESEM. This treatment could be considered for the prevention of dental cavities.

Authors’ Contributions

Ghada Abdul Salam Ibrahim contributed to the literature search, data collection, taking the results, analysing them, and writing them down in the research. Eaman Ali Al-Rubaee, and Maha Jamal Abbas, contributed to the work in terms of designing the work plan.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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