The Impact of CO_2 Laser and Hydroxyapatite Nano Particles on Dental Enamel

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Abstract: ***Background:*** ***Laser technology holds great promise for medical and dental applications and carbon dioxide (CO2) laser is still one of the most beneficial type. A laser beam is created from a substance known as an active medium, which when stimulated by light or electricity produces photons of a specific wavelength. This beam results in an interaction between light and biological constituents of tissues that are converted into heat; ending with structural and chemical changes in tissues. Dental caries is a dietary carbohydrate-modified bacterial infectious disease. The basic mechanism of dental caries is demineralization through the acids attack. Lasers are expected to be one of the most promising new technical modalities for the treatment of dental diseases. Nanotechnology is "the manipulation of matter on the molecular and atomic levels. The developed interest for nanotechnology in many fields, is producing interesting and imminent applications as tissue repair and replacement especially in bones and dental mineralized tissues. Aim of the study: this study was conducted to test the impact of CO_2 laser and Hydroxyapatite nanoparticles on the change in chemical composition of enamel and the morphological changes in enamel ultra-structure. Materials and Methods: Teeth samples in this study consisted of 66 maxillary first premolars, divided into six groups: one control group and five study groups, each group consisted of 11 teeth; one tooth for Scanning Electron Microscope examination (SEM), while other 10 teeth for Energy dispersive spectroscopy (EDS) analysis.A position of circular window on the buccal surface of each tooth was standardized. To induce caries lesion on enamel surface, pH cycling procedure was followed. Lasing was carried out using CO2 laser system at specific power, time and mode. Hydroxyapatite nanoparticles used in the study was 20nm, the concentration was determined to be 10%. The weight percentages (wt%) of Calcium, Phosphorus, Oxygen were determined using EDS analysis to evaluate the change in chemical composition of enamel; SEMwas used to demonstrate the morphological changes in enamel ultra-structure. Results: For all groups, the data obtained by (EDS) analysis revealed that for both Calcium (Ca) and Phosphorus (P), the mean atomic percentage was reduced after demineralization and after laser irradiation. An increase in the atomic percentage for both elements after treatment with other agents was noticed, with the maximum value recorded for the group treated with Laser + hydroxyapatite nanoparticles. For Oxygen (O), the result was opposite to that of (Ca) and (P). Statistical analysis werehighly significant ($p<0.01$) for all of the three elements. Examination of enamel surface using SEM revealed an ultrastructural change had occurred beginning with loss of enamel normal architecture after demineralization. After CO2 laser irradiation, cracks and melted and recrystallized areas were noticed. After treatment with hydroxyapatite nanoparticles, hydroxyapatite nanoparticles + CO2 laser and CO2 laser + hydroxyapatite nanoparticles most of micropores were occluded and surface defects were reconstructed. Conclusion: Treatment of enamel surface with CO_2 laser + hydroxyapatite nanoparticles gave the best result regarding EDS analysis and SEM examination, so such treatment could be considered as a method of preventing demineralization and encouraging remineralization of enamel.***

Keywords: CO_2 laser, hydroxyapatite nanoparticles, EDS analysis, SEM examination

1. Introduction

Developments in physics at the beginning of the twentieth century laid the foundation for laser theory postulated by Albert Einstein, culminating in the invention of this special form of light in 1960. Soon thereafter, researchers began to explore possible applications of laser technology in medical and dental treatment [1].Developments in technology and differing wavelengths are creating a host of innovative treatments using various dental lasers [2].Carbon dioxide laser (CO_2 laser) was one of the earliest gas lasers to be developed, invented by Kumar Patel in 1964, and is still one of the most useful [3].

Enamel forms a protective covering of variable thickness over the entire surface of the crown of the tooth. It is the hardest tissue in the human body, it acts as a semi permeable membrane permitting complete or partial passage of certain molecules [4] - [5]. Dental cariesis an infectious microbiological disease of the teeth result in localized dissolution and destruction of the calcified tissue [6].Dental caries is a multifactorial disease related to the interaction of the bacteria on the tooth surface, the dental plaque or oral biofilm, the diet specifically fermentable carbohydrate components of the diet, which are fermented by the plaque microflora to organic acids, and the teeth acting together over time [7].Although dental caries is a preventable disease, it is still common and remains a public health problem, so the aim of modern dentistry is the early prevention of tooth decay, rather than invasive restorative therapy [8].

One of the potentially effective preventive measures is the use of laser. Increasing knowledge about the interaction of laser with hard tissue was tested and good results relating to the inhibition of incipient caries were observed [9-10].It has been demonstrated that treatment with several kind of lasers can reduce the rate of subsurface demineralization in enamel, and a variety of explanations have been given for this process. Nevertheless, the real mechanisms of caries inhibition by laser remain unclear. It could be due to decreasing enamel permeability to chemical agents due to melting and physical fusion of the enamel surface microstructure [11] - [12].

Nanotechnology is "the manipulation of matter on the molecular and atomic levels". Nanometer is 10^{-9} meter, or one billionth of a meter. Nano dentistry creates incredibly useful structures from individual atoms or molecules (nanoparticles), which provides a new alternative strategies in prevention and treatment of dental caries, specifically in the remineralization of initial dental caries. Hydroxyapatite nanoparticles material is attracting interest as a biomaterial for use dentistry due to its outstanding properties and interesting applications as tissue repair and replacement especially in bones and dental mineralized tissues [13].

2. Materials and Methods

Teeth samples in this study consisted of 66 maxillary caries free first premolars, that were divided into six groups: one control group and five study groups, each group consisted of 11 teeth; one tooth for Scanning Electron Microscope examination (SEM), while other 10 teeth for Energy dispersive spectroscopy (EDS) analysis as follow:

Group 1: Control teeth.
Group 2: Teeth subjected toPH cycle.
Group 3: Teeth treated with CO_2 laser.
Group 4: Teeth treated withHydroxyapatite nanoparticles.
Group 5: Teeth treated withCO_2 laser then Hydroxyapatite nanoparticles.
Group 6: Teeth treated with Hydroxyapatite nanoparticles then CO_2 laser.

A position of circular window on the buccal surface of each tooth was standardized. The window of each tooth was ground and polished to provide a flat surface of each tooth for EDS analysis and SEM [14]. To induce caries lesion on enamel surface, pH cycling procedure was followed by preparation of demineralizing and remineralizing solutions and adjustment of pH [15].

Lasing was carried out using CO_2 laser system, according to a special equation [16].The exposure was perpendicular to enamel surface and the power density was (3W/cm^2) [17].
Nano-Hydroxyapatite used in this study was hydroxyapatite nano powder Ca_{10} $(PO4)_6$ $(OH)_2$ with 99% in purity, white in color, 20nm. The concentration of hydroxyapatite nanoparticles solution in this study was determined to be 10%. [18-19].

Representative one specimen from each group was selected for SEM sample preparation to examine the morphological changes occur in enamel surface by scanning it with a focused beam of electrons [20]. The selected teeth were gold coated by placing them in a vacuum system coating machine in order to enable or improve the imaging of samples [19-21].The weight percentages (wt%) of Calcium (Ca), Phosphorus (P), Oxygen (O) were determined using EDS, as it's an elemental analysis or chemical characterization of a sample. It relies on an interaction of some source of X-ray excitation and the sample [22].

3. Result

3.1 Energy Dispersive Spectroscopy (EDS)

The data obtained by (EDS) analysis for all groups regarding the atomic percentage of Oxygen (O), phosphorous (P) and Calcium (Ca) were shown in Table (1).For both (Ca) and (P), the mean atomic percentage was reduced after demineralization, a further slight reduction was noticed after treatment with laser for both elements. An increase in the atomic percentage was noticed for both elements after treatment with (hydroxyapatite nanoparticles, hydroxyapatite nanoparticles + Laser, and Laser + hydroxyapatite nanoparticles) as compared to demenrilization stage. The maximum value for both (Ca) and (P) was recorded with Laser + hydroxyapatite nanoparticles group. For (O), result revealed that the atomic percentage increased after demineralization and a further increase was observed in the lased group. A reduction in the atomic percentage of (O) was noticed in the remaining groups. Statistical analysis werehighly significant ($p<0.01$) for all of the three elements.

Table 1: Descriptive (mean and standard deviation) of Energy Dispersive Spectroscopy (EDS) of the atomic percentages for Oxygen (O), phosphorous (P) and Calcium (Ca)for each groups with a statistical test

Group	*Mean*			*±SD*		
	O	*P*	*Ca*	*O*	*P*	*Ca*
Sound	60.24	15.66	24.11	1.08	1.09	1.20
Demineralization	64.18	14.86	20.96	.94	.97	1.05
Laser	66.30	14.05	19.65	.78	.97	1.05
Hydroxyapatite Nanoparticles	63.08	15.04	21.89	.85	.89	1.24
Hydroxyapatite nanoparticles +Laser	57.76	15.37	26.87	1.39	.71	1.45
Laser+ Hydroxyapatite Nanoparticles	55.52	15.91	28.57	1.48	.70	1.53
ANOVA	O	F=131.414P-value=0.000**				
	P	F=20.894P-value=0.000**				
	Ca	F=79.828P-value=0.000**				

**Highly significant

3.2 Scanning Electron Microscope (SEM)

For sound enamel surface Figure (1), normal, smooth and intact enamel surface structure devoided from any alteration was noted before demineralization; normal perikymata appear to run in parallel lines. Some holes could be seen. The structure of enamel surface after demineralization was changed in Figure (2) enamel lost its normal architecture and the prisms showed irregularity. Numerous voids and micropores apparently seen on the enamel surface. Figure (3) showed the SEM image of enamel surface irradiated with CO2 laser. It showed absence of normal enamel perikymata, cracks, craters, micro spaces formation andirregular melted and recrystallized areas. In Figure (4) the changes in enamel surface structure after treatment with hydroxyapatite nanoparticles were demonstrated. Morphological changes were observed by the presence of amorphous, globular and crystalline structures that occlude the micropores created after demineralization. In the image that demonstrated the structural changes created by treatment with hydroxyapatite nanoparticles + CO_2 laser Figure (5), most of the micropores were hidden and occluded. The hydroxyapatite nanoparticles appeared to be melted, recrystallized, and trapped inside the pores forming a plug. The SEM of enamel surface treated with CO_2 laser+ hydroxyapatite nanoparticles Figure (6) showed that the surface defects that were produced by laser irradiation were reconstructed by the deposition of a layer of hydroxyapatite nanoparticles.

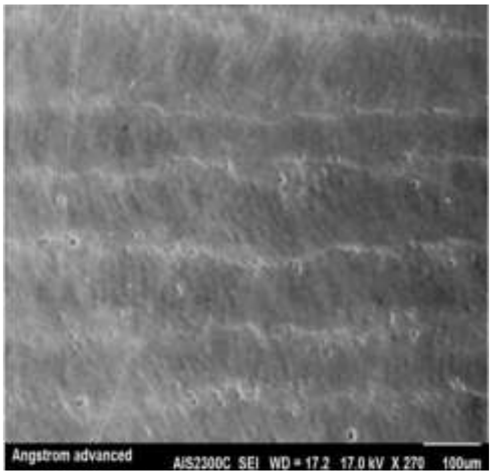

Figure 1: SEM for normal sound enamel surface

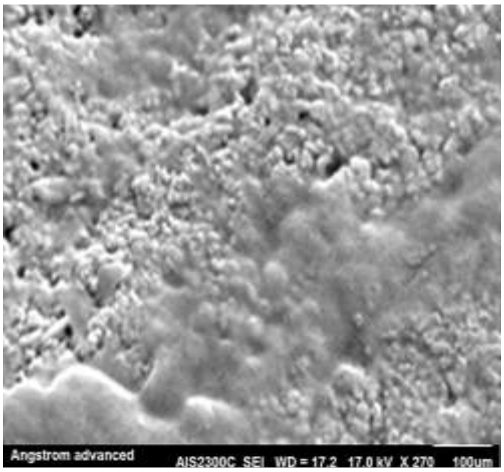

Figure 2: SEM for demineralized enamel surface

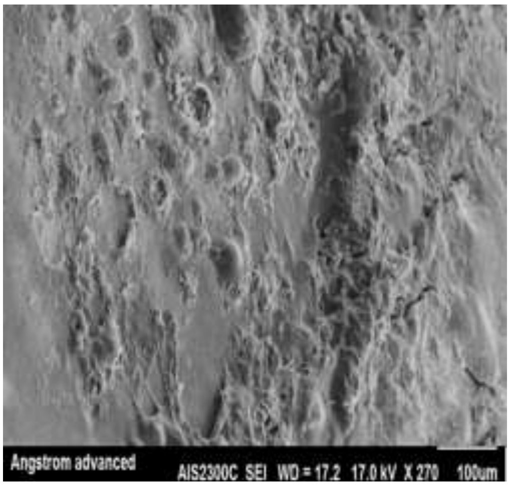

Figure 3: SEM for enamel surface of toothtreated with CO2 laser

Figure 4: SEM for enamel surface of tooth treatedwith hydroxyapatite nanoparticles

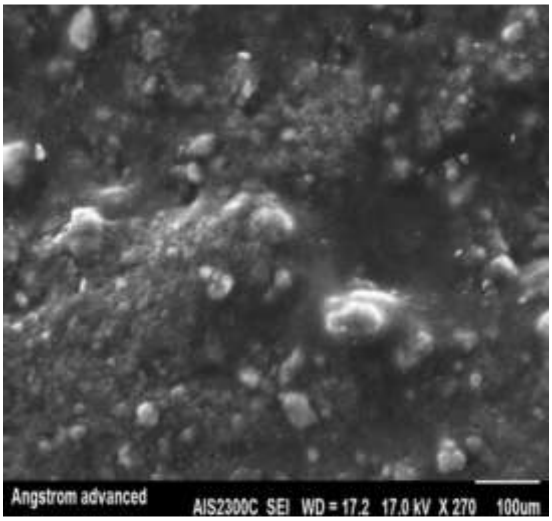

Figure 5: SEM of enamel surface of tooth treatedwith hydroxyapatite nanoparticles + CO_2 laser

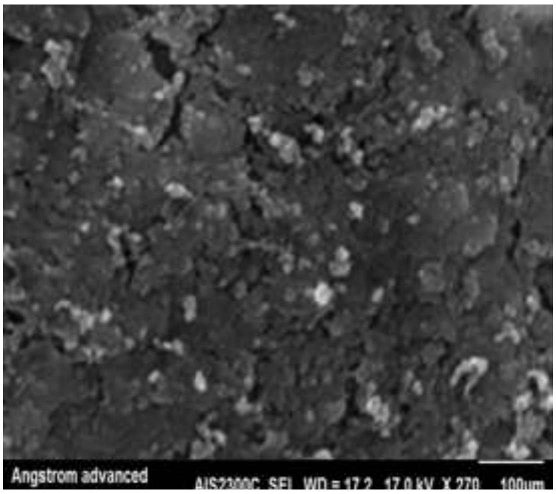

Figure 6: SEM for enamel surface of tooth treated with CO_2 laser+ hydroxyapatite nanoparticles

4. Discussion

This study was conducted to investigate the combined effect of CO_2 laser and hydroxyapatite nanoparticles on enamel structure. The data obtained from EDS analysis showed a reduction in the mean atomic percentage of calcium and phosphorous after demineralization when compared to the control (sound teeth). This result was confirmed by SEM micrograph for demineralized enamel surface that revealed numerous voids and micro spaces after pH cycling, as an indication of enamel demineralization and initiation of carious lesion because any reduction in PH of the surrounding environment below critical PH (5.5), will create an acidic medium that causes outward movement of the minerals of tooth mainly calcium and phosphorous leaving behind micro pores and reducing hardness [23].

For the group treated with laser alone, the mean atomic percentage for both calcium and phosphorous showed a reduction when compared to the demineralization stage and sound teeth stage. This is probably because when teeth irradiated with laser, an interaction between light energy and biological constituents of enamel will occur and this interaction will be converted into heat causing changes in the chemical constituents of enamel. Melting and re-solidification processes will occur with formation of micro spaces (holes) as a result of evaporation of water from the enamel matrix [24]. This was confirmed by SEM micrograph. The melting process and the formation of micro spaces might affect calcium and phosphorous ions level in the outer enamel surface; however, further studies are needed to be carried out regarding this point.

For the group treated with hydroxyapatite nanoparticles, EDS analysis revealed that the mean atomic percentage for calcium and phosphorous ions was increased as compared to demineralization stage, this may be attributed to that hydroxyapatite nanoparticles penetrate the enamel micro spaces, and act as a template in the precipitation process and will continuously attract a large amount of calcium and phosphorus from the remineralizing solution to the enamel surface to fill the vacant positions of the enamel crystals and significantly promoting the remineralization effect [25]. This was confirmed by the SEM micrograph that revealed amorphous, globular and crystalline structures that precipitated on enamel surface and occlude the micro spaces. For the group treated with hydroxyapatite nanoparticles followed by laser, EDS analysis revealed an increase in the mean atomic percentage of calcium and phosphorous ions, as aresult of a synergistic effect ofboth laser and hydroxyapatite nanoparticles. Another explanation is that when enamel surface irradiated with laser, laser will favor ion linking of the mineralizing agent in that area [26-27]. This is also confirmed by SEM micrograph that revealedmost of the micro spaces were occluded by hydroxyapatite nanoparticles that appeared to be melted, recrystallized, and trapped inside the micro spaces forming a plug. For the group treated with laser followed by hydroxyapatite nanoparticles, EDS analysis revealed a sharp increase in the mean atomic percentage of calcium and phosphorous ions as compared to the demineralization stage. The possible explanation is that when laser irradiation was applied first, it creates micro spaces (holes) which would facilitate the ions incorporation [28], so these micro spaces would be filled with hydroxyapatite nanoparticles after their application. The result was confirmed by SEM micrograph provided further conformation for this finding, which revealed that most of the surface defects that were produced by laser irradiation were reconstructed by the deposition of a layer of hydroxyapatite nanoparticles.EDS analysis for the Oxygen, demonstrated that the atomic percentages increased and decreased in adverse direction to that of calcium and phosphorous, which is difficult to be explained because the exact chemical composition of enamel crystals that would be formed after application of different mineralizing agents is not exactly known. As a result, it is advocated in future studies to use advanced technologies to reveal the crystallographic and chemical changes that takes place after application of different agents to enamel surface.

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