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The Effect of CO_2 LASER, Hydroxyapatite and Iron oxide Nano Particles on Human Dental Enamel and *Streptococcus Mutans* (*In Vitro study*)

A Thesis
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By
Farah Abdul Razzaq Mahmood Al-Bazzaz
B.D.S., M.Sc.

Supervised by

Assistant Prof.
Dr. Nada Jafer MH. Radhi
B.D.S, M.Sc., Ph.D.
(Preventive Dentistry)

Assistant Prof.
Dr. Kadhim A. Hubeatir
B.Sc., M.Sc., Ph.D.
(Laser Technology)

Baghdad-Iraq
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Abstract

Background: Specific laser technologies are expected to become essential components of medical and dental practice for targeting specific cells, pathogens, or molecules and a combination of diagnostic and therapeutic uses.
The field of Nanotechnology is attracting attention due to the outstanding properties and imminent applications of these nanoparticles in tissue repair and replacement. Laser and nanotechnology are expected to be one of the most promising new technical modalities for the treatment of dental diseases.

Aim of the study: to test the effect of carbon dioxide laser, hydroxyapatite nanoparticles (HANPs) and iron oxide nanoparticles (IONPs) on microhardness, chemical composition of enamel and morphological changes in enamel ultra-structure. Also to test the sensitivity of *Streptococcus mutans* to carbon dioxide laser and different concentrations of both type of nanoparticles.

Subjects, materials and methods: The sample was consisted of 89 maxillary first premolars, divided into control and study groups for microhardness testing, scanning electron microscope examination (SEM) and energy dispersive spectroscopy (EDS) analysis. A circular window on the buccal surface of each tooth was prepared. Teeth were exposed to carbon dioxide laser at specific parameters. The concentration of hydroxyapatite nanoparticles used was 10%, while for iron oxide, it was 12.5%. Enamel microhardness was measured for normal enamel, after pH cycling and after treatment with different agents. Change in the hardness was calculated with a special equation. The atomic percentages of calcium, phosphorus, oxygen were determined using energy dispersive spectroscopy analysis to evaluate the change in chemical composition of enamel; scanning electron microscope was used to demonstrate the morphological changes in enamel ultra-structure. The radiation effect of

carbon dioxide laser was tested in different times and powers on the viable counts of *Streptococcus mutans*. Standardized suspensions of bacterial growth were inoculated in seven sterile screw capped bottles, one of them was control and the other six were exposed to carbon dioxide laser. After radiation, 0.1 ml of the suspension was spread over the surface of agar plates then incubated. The microbiological assay was carried out by counting the colony forming unit/ milliliter (CFU/ml). Testing the sensitivity of *Streptococcus mutans* to different concentrations of hydroxyapatite and iron oxide nanoparticles compared with chlorhexidine 0.2% as a control positive and de-ionized water as control negative was carried out using agar well diffusion technique. Zone of inhibition is measured across the diameter of each well. The data of the current study was analyzed using statistical package for social science (SPSS version 21) and R 3.3.2.

Results: There was a reduction in enamel microhardness after demineralization as compared to sound teeth for all groups. After treatment with different agents, a marked increase in microhardness for all groups was found with a highly significant difference, with the maximum increase was in the group treated with laser+ hydroxyapatite nanoparticles and the group treated with laser+ iron oxide nanoparticles. Treatment of teeth with laser+ iron oxide nanoparticles caused the highest change in enamel microhardness, while treatment with iron oxide nanoparticles resulted in the lowest. The data obtained by energy dispersive spectroscopy analysis revealed that for both calcium (Ca) and phosphorus (P), the mean atomic percentage was reduced after demineralization and laser radiation. An increase in the atomic percentage for both elements after treatment with HANPs, HANPs+laser, laser+HANPs groups 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 Calcium and Phosphorous. Statistical analysis were highly significant for all of the three elements. Scanning electron microscope examination revealed ultrastructural

changes occurred beginning with loss of enamel normal architecture after demineralization. After laser radiation, cracks, melted and recrystallized areas were noticed. After treatment with other tested agents, most of micro pores were occluded and enamel surface defects were reconstructed. Exposing *Streptococcus mutans* to carbon dioxide laser, resulted in a reduction in colony forming unit/ml with a highly significant difference and there was an increase in the percentage of bacterial killing with increasing exposure times and powers, and the maximum value was recorded in the group radiated with 4Watt for 60 seconds. There was an increase in the values of mean of inhibition zone for last three concentrations of iron oxide nanoparticles with a highly significant difference among groups. When making multiple comparisons of the inhibition zones of iron oxide nanoparticles between groups, findings showed that the inhibition zones of 17%, 20% and 22.5% were more than all other concentrations that had no inhibition zones with a significant difference.

Conclusions: Regarding microhardness, best results was for the teeth treated with laser+ hydroxyapatite and laser+ iron oxide nanoparticles and this was supported by energy dispersive spectroscopy analysis and scanning electron microscope examination. Carbon dioxide laser was found to have antibacterial effect on *Streptococcus mutans*. *Streptococcus mutans* were not sensitive to hydroxyapatite nanoparticles, but they were sensitive to iron oxide nanoparticles and the sensitivity increased with increasing concentration.