The Effect of Nd:YAG Laser and Casein-Phosphor-Peptide-Amorphous Calcium Phosphate with Fluoride on Dental Enamel De-mineralization: In vitro study

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ABSTRACT
The aim of the study was to evaluate a new treatment modality to decrease dental enamel demineralization via combing the effect of pulsed Neodymium Yttrium Garnet (Nd:YAG) laser (1064 nm) and re-mineralizing paste (CPP-ACP containing sodium fluoride) regarding the tooth pulp chamber temperature elevation, calcium concentration dissolution from enamel surfaces and the dental enamel absorption of methylene blue stain by assessing (R red, G green, B blue) values. Sixty human permanent premolars were used for measuring calcium concentration dissolution and (R, G, B) values. They randomly divided into six groups: the negative control group (A) received no treatment, and the positive control group (B) where the re-mineralizing paste was applied without laser irradiation. For the groups (C), (D), (E) and (F) the (1064 nm) pulsed Nd:YAG laser was used in two dose settings. The lower laser dose setting at (60 mJ) for the C and D groups, and the higher laser dose setting at (170 mJ) for E and F groups respectively. Groups D and E in addition to laser irradiation the re-mineralizing paste was applied.

The atomic absorption photometry test results showed that groups C, D, and E were statistically different compared to the groups A and B (p<0.001) in the reduction of calcium dissolution, Whereas Groups E and F shown non-significant reduction in calcium dissolution compared to control groups.

Twenty teeth were used to assess the temperature elevation during irradiation using a K-type thermocouple at 37 ± 0.5°C, divided for ten teeth for each irradiation settings. The maximum temper of temperature test showed that the maximum temperature increases for group E was very high significant difference (p<0.05) over that of groups C.

The (R, G, B)s value test showed that groups C and D having lower affinity to absorb methylene blue stain, and they are statically significant different compared to the groups A and B, while groups E and F showing a higher affinity to absorb methylene blue stain compared to control groups.

KEYWORDS
pulsed Nd:YAG laser, (R,G,B) values, enamel demineralization.

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INTRODUCTION
Dental caries is continuous most prevalent disease during childhood and Adolescence (1). Dairy products including milk, milk concentrates and cheese have long been known to exhibit anti-caries activity where the responsible components being identified as casein, calcium and phosphate. The repair of early tooth enamel lesions has been recently demonstrated by tryptic phospho-peptides derived from milk caseins that associate with amorphous calcium phosphate forming stable complexes (2). In addition to fluoride, a casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and fluoride containing CPP-ACP pastes have also been recommended for caries

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prevention and enamel remineralization \(^{(3,4,5)}\). Since the 1960s, it has been consistently confirmed that lasers can significantly increase the acid resistance of enamel, and when associated with fluoride, a significant synergism between them has been shown in the reduction of enamel solubility \(^{(6)}\).

The use of lasers in caries prevention was first suggested in 1972 using a ruby laser, since then, many investigations related to the application of other lasers, such as argon, CO\(_2\), and Nd:YAG lasers, in the area of preventive dentistry, have been carried out and laser irradiation has been proposed as an adjunct to conventional caries prevention therapies\(^{(7)}\). Combing the effect of laser irradiation with re-mineralizing agent therapies, conducted that can be increase the resistance of tooth structure to mineral loss from the organic acids involved in dental caries\(^{(8,9)}\). Recently there are no studies addressing the combining the effect pulsed Nd;YAG laser and CPP-ACP with sodium fluoride paste on decreasing dental enamel de-mineralization, either the evaluation the effect of methylene blue stain of laser irradiating dental enamel surfaces. New approaches for caries prevention by using lasers effect combined re-mineralizing agent providing calcium and phosphate in addition to fluoride for increasing the tooth mineral resistance needed, this study aimed to evaluate the effect of energy densities of pulsed Nd:YAG laser \((\text{Han’s Laser system Pulsed Nd:YAG laser, PB 80, manufactured by Han’s Laser Technology, Co. Shenzhen, China)}\),60mJ \((0.6 \text{ W})\), and 170 mJ\((1.7 \text{ W})\), pulse width 1 ms, irradiated three times for three seconds with cooling interval 15s. Re-mineralizing paste was applied on the samples for three minutes and repeated four times, then exposed for laser irradiation and acid challenge by incubate them at controlled temperature of 37°C in 3 ml of Acetate-buffer solution pH value of 4.5 for 48 hours. The solution then analysed by an atomic absorption spectrophotometer in Ibn Sina Co. Iraq.

**Thermal test**

The tooth was used in a hot water bath. The K-type of Basic accuracy \((0.05\% \text{ rdg±0.3 Celsius)}\) thermocouple was inserted into pulp chamber on the opposite site to the irradiated widow the thermal grease of thermal conductivity \(>0.6\text{ W/m-k} \) injected inside the pulp chamber for better contact between the thermocouple and the dentine surface.

**Colour test**

The samples were immersed in 2% methylene blue solution for one minute. The access removed by gauze, the each sample photographed by using high Nikon camera \((D3300,Tokyo,Japan)\) and computer image processing software Adobe Cs2 2014 on windows 8 the \((R,G,B)\) values were measured to assess the dental enamel colour changing.

**RESULTS**

The temperature test results for each sample of group C and E respectively analysed by descriptive statistics, and Student’s t-test showing very high statistical significance group E over group C \((p<0.05)\). Thicknesses measurements of both enamel and dentine measurement values for groups C and E showing no statistical significance between the tested groups \((\text{Table 2})\).

Atomic absorption analysis done by using ANOVA test that shows a very high significant difference \((p<0.0001)\) between and within tested groups for of calcium concentration in part per million.

**MATERIAL AND METHODS**

Eighty sound extracted human first premolars extracted for orthodontic purposes 14 - 27 years used in this vitro study. Sixty teeth were used for ions analysis and digital photographic colour value analysis. Sample surfaces were examined with the stereomicroscope X10 to check surfaces were free of detectable white spot lesions. The teeth were washed and debride of attached tissue by curette and ultrasonic. The teeth were then polished with fluoride-free prophylaxis paste. All prepared teeth were stored in deionized water with 0.1% thymol solution at 4°C. The solution was changed on weekly basis until the teeth were used.

The samples coated with nail varnish leaving circular window of 4mm in diameter. Then they split into two halves mesio-distally and horizontally at the cemento-enamel junction by disc saw mounted on low speed handpiece under running distilled water.
Calcium concentrations were decreased for lower laser doses, but the significant reduction in lower laser dose with MI paste. The colour (R, G, B) values analysis measurements by ANOVA test shows a very high significant difference (p<0.0001) for (R, G, B) values for all samples of each group respectively. Groups C and D are significantly different in absorbing methylene blue stain in comparison to other groups.

**DISCUSSION**

The temperature was measured for group C (60 mj), and group E (170 mj) to insure that the used parameters were not harmful for the dental pulp tissue. The light should be transformed into heat in an efficient manner without causing thermal damage to the adjacent or subjacent tissues, the maximum temperature inside the pulp recorded for GC and GE were 2.5°C and 4.7°C respectively, with maximum average enamel and dentine wall thicknesses measured 3 mm and 3.1 mm respectively. Both temperature records of GD and GE seems fall within the safety limits, so there is agreement with the study. According to Beer–Lambert’s law (the basic law of thermodynamics), which states that there is an inverse relation between the thickness of the matter and the temperature deposited on it: dQ = m c where (dQ) is the heat content, (m) represents tooth mass, (c) is the heat capacity and (dT) is the linear change in the temperature. A hole was made opposite the lasing area, so that the thermocouple inserted inside the pulp chamber was isolated by the tooth structure, and may not detect the heat dissipated to that medium. A thermal compound was used inside the pulp chamber to prevent a gap formation between the thermocouple and the dentin.

The acetate acid present in this solution was one of the three main acids produced by cariogenic plaque and, therefore produce the required demineralization pattern. The lower Calcium concentrations observed in group C (60 mJ), and group D (60mj with remineralizing paste), with reduction in (Ca²⁺) in ppt. percentage of (25.7%, 21.75%) for group C with respect to negative and positive controls group respectively, While group D shows reduction percentage of calcium atoms concentration (33.34%, 29.8%) as compared with negative and positive controls groups respectively. The reduction of Calcium dissolution is probably due to a reduction of permeability of enamel, a lower penetration of acid inside the underneath layers, and prominent chemical and mineral content changes, this is agree with the pervious study. While the GE(170 mj) and GF(170mj with MI paste plus) they are not showing a significant reduction in calcium concentration compared to GC and GD this may be due to the energy dose that used for (GE and GF) was high to producing enamel acid resistance.

R, G, B values are the three basic component of digital image. The colour assessment widely used in dentistry, and measurement of RGB value newly introduced as a new method for demineralization detection, using a digital camera is an alternative way to use a color meter, so it is easy and low cost technique. The analysis used in this experimental research to show if there are any deference of tested groups to absorb methylene blue stain, and hence indicate the effect of acid dissolution on dental enamel samples. The results show a highest R,G and B values for GC and GD as compared to the groups A and B. These high values indicate brighter color and therefore less permeability of enamel surfaces, which means less demineralization, so there is agreement with other proposal. However R,G,B values for groups E and F were not significantly different from control groups that indicating a higher absorption of methylene blue stain. Also this test results may agree with the atomic absorption test results for groups C and D that showing lesser calcium concentration dissolution, that means the less demineralized enamel surfaces are poorly absorb stain.

**CONCLUSION**

The pulsed Nd:YAG laser of (60 mj, 11j/cm²) combing with Casein- Phosphor-Peptide-Amorphous Calcium Phosphate with Fluoride decreasing dental enamel demineralization. The temperature elevation during using pulsed (1064 nm)Nd:YAG laser at (60 mj and 170 mj) was safe to the dental pulp, and the colour analysis test showed decrease in stain absorption of enamel surfaces treated with (60 mj) combined with the remineralizing paste.

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### Table 1: Student’s t-test of thermal records

<table>
<thead>
<tr>
<th>Group</th>
<th>Means</th>
<th>S.D</th>
<th>t-test</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2.22</td>
<td>0.131</td>
<td>35.77</td>
<td>0.0001</td>
<td>VHS</td>
</tr>
<tr>
<td>E</td>
<td>4.49</td>
<td>0.152</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Student’s t-test for thickness measurement between (C) and (E) groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Means</th>
<th>S.D</th>
<th>t-test</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2.92</td>
<td>0.079</td>
<td>0.448</td>
<td>0.66</td>
<td>NS</td>
</tr>
<tr>
<td>E</td>
<td>2.94</td>
<td>0.117</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Multiple comparisons indicate significant differences in level among the groups for (Ca) in ppt.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Mean ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>108.85± 11.28</td>
</tr>
<tr>
<td>Group B</td>
<td>103.36± 8.58</td>
</tr>
<tr>
<td>Group C</td>
<td>80.87± 2.6</td>
</tr>
<tr>
<td>Group D</td>
<td>72.56± 2.078</td>
</tr>
<tr>
<td>Group E</td>
<td>96.7± 2.1</td>
</tr>
<tr>
<td>Group F</td>
<td>89.45± 3.6</td>
</tr>
</tbody>
</table>

Figure 1: (R,G,B) values for all samples of each group

REFERENCES