International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.6, pp 937-944, 2017

# Effect of $CO_2$Laser and Casein Phosphopeptide- Amorphous Calcium Phosphate paste on Morphological and Chemical Changes of Initial Caries-Like Lesion of Permanent Teeth

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**Abstract :** The irradiation of teeth with laser in combination with remineralizing agent revealed higher caries resistance. This study aims to investigate the effect of laser and CPP-ACP agent in remineralizing initial caries-like lesion using SEM and EDS analysis. Twenty four teeth (upper first premolar) were divided into control and study groups, the study group was subdivided into five groups (pH_cycling, laser, CPP-ACP, CPP-ACP followed by laser, Laser followed by CPP-ACP), each group included four teeth, one for SEM and three for EDS analysis. Surface temperature change equation was used to determine the maximum power that enhances morphological and chemical changes in the enamel surface without thermal damage. For both calcium and phosphorus the mean atomic percentages were reduced after demineralization (pH_cycling), then after the application of laser both calcium and phosphorus revealed slight reduction. After application of remineralizing agent (CPP-ACP) both calcium and phosphorus the mean atomic percentages increased and further increase of both calcium and phosphorus the mean atomic percentages was recorded when a combination of CPP-ACP and laser was used. A maximum increase of the mean atomic percentage of both elements was recorded when the laser was applied first followed by CPP-ACP agent. The combination of laser therapy followed by CPP-ACP application is recommended as efficient preventive measure for remineralizing initial caries – like lesion in permanent teeth. Also the use of 0.852 watt for 5 seconds was efficient in enhancing morphological and chemical changes in the enamel surface without thermal damage.
**Keywords :** Enamel; EDS; $CO_2$ laser; SEM; CPP-ACP.

## Introduction

Dental caries are still considered the most prevalent disease during childhood and adolescence [1,2]. In spite of decline in its incidence worldwide in recent decades. The disease has become more selective affecting mostly children particularly in families of low socioeconomic states[3,4] and in immunocompromised children[5].

The aim of modern dentistry is the early prevention of tooth decay, rather than invasive restorative therapy. Various therapeutic agents have been introduced to restore early enamel lesions by enhancing their remineralization. Fluoride (in different forms) has been used for years as the most common substance to prevent dental caries[6]. Casein phosphopeptide-amorphous calcium phosphate is considered as other preventive substance that has drawn considerable attention[7,8,9,10]. Casein phosphopeptides (CPP) are peptides derived from the milk protein casein that are complexed with calcium and phosphate. Its role in remineralization of early carious lesions and increasing enamel microhardness has been confirmed by several studies[11,12,13,14].

Another adjunct method in dental caries prevention is laser irradiation[6,15]. Since 1960s it had been demonstrated that the use of lasers can reduce the rate of sub surface demineralization of enamel[15,16,17,18,19,20,21]. Combination of laser irradiation with other remineralizing agents have been shown to further reduced enamel demineralization and improving its microhardness[15,21].

In the light of these reflections, this study designed in order to assess the effect of $CO_2$ laser irradiation and CPP-ACP application on morphological features and chemical of initial caries- like lesion of permanent teeth.

## Materials and Methods

Twenty four upper first premolars extracted for orthodontic reasons were washed with de-ionized water, and to remove any debris each tooth was wiped with acetone, then stored in 20 ml of 0.1% thymol solution (wt./Vol) to prevent microbial growth. Teeth samples were kept in refrigerator at 4˚C until use[22]. Samples were cleaned and polished using a slowly rotating conventional hand piece and de-ionized water and rubber cup with non fluoridated pumice. Teeth were wiped with acetone soaked cotton to remove any grassy pumice. Each tooth surface was examined under magnifying lens under good light to check if any crack or defect might be present

Twenty four teeth were divided into control and study groups, the control group included four teeth, Sound group (control) were stored in de-ionized water at temperature of 37˚C in the incubator.

The study group was subdivided into five equal groups(four teeth in each group). The first study group was teeth that subjected to pH_cycling to initiate artificial caries by following the procedure designed by Featherstone et al.[23]. The second study group was teeth that treated by CPP-ACP for four minutes by topical coating (8mg) placed in contact directly to the windows of buccal surfaces, then each tooth was rinsed for two minutes with de-ionized water. Teeth were re-stored in de-ionized water at temperature of 37˚C in the incubator, for the next day this procedure was repeated daily for one week. The third study group was teeth that treated by $CO_2$ laser with power of 0.852 watt for five seconds and the laser was directly irradiated the window on buccal surface. The fourth study group was teeth that treated by CPP-ACP followed by laser and the fourth study group was teeth that treated by Laser followed by CPP-ACP according to the procedure discuss above.

Then one sample from each group was tested by scanning electron microscopy, and three samples from each group were tested by energy dispersive spectroscopy, see figure (1).

Data analysis was performed by using SPSS (statistical package for social science) (version 21). Statistical tests used were two-way ANOVA, the confidence limit was accepted at 95% ($p < 0.05$).

Surface temperature change equationwas used todetermine the power of laser that irradiated the enamel surface without any thermal damage[24] :

$$H=\left\{\frac{P(1-r_f)}{A}\right\} \quad (1)$$

$$T_{z,t}=\frac{2H}{K}\left\{(xt)^{\frac{1}{2}}\mathbf{ierfc}\left(\frac{z}{2\sqrt{xt}}\right)\right\} \quad (2)$$

Where the "integral of the complimentary error function", ierfc , means:

$$\mathbf{ierfc}(\mathbf{u})=\frac{e^{u^2}}{\sqrt{\pi}}-\mathbf{u}[1-\mathbf{erf}(\mathbf{u})] \quad (3)$$

Where H is the surface power density, T is the temperature, x is the thermal diffusivity, t is the time, z is the Depth, A is the area, P is the power, and rf is the reflectivity.

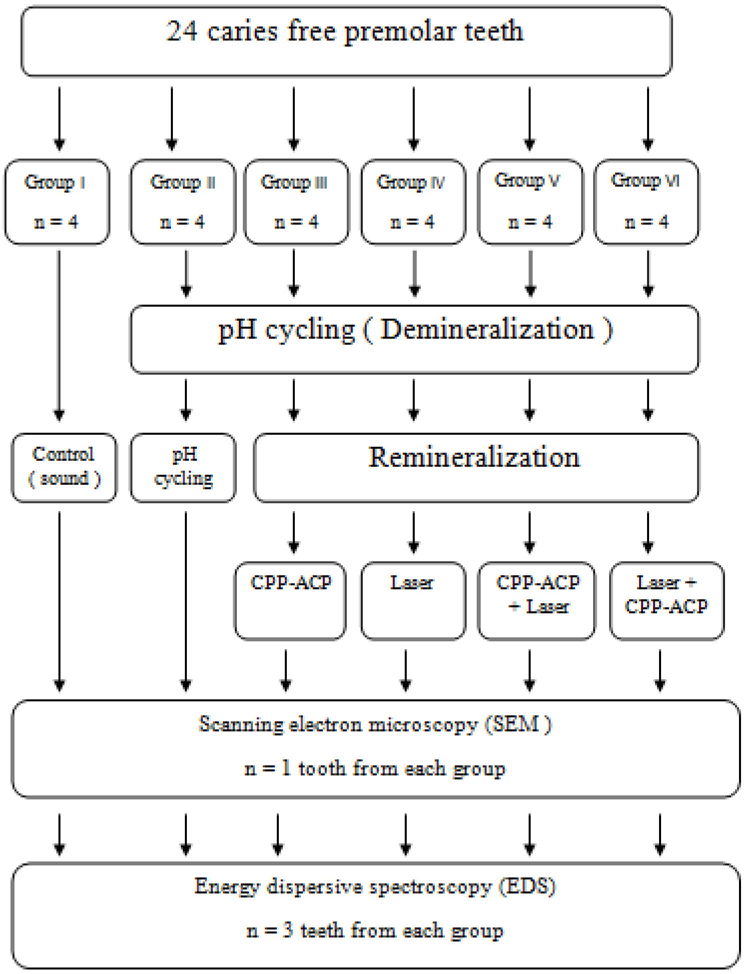



**Figure (1): Experimental Design**

## Results

### Energy Dispersive Spectroscopy analysis

The experimental data obtained by EDS analysis for all groups regarding the atomic percentages of O, P and Ca are displayed in Table (1). The atomic percentage (at %) was used instead of weight percentage (wt %), because it is more convenient to consider the difference in the number of atoms, rather than their weight. In this way, we can better correlate any changes in tooth composition directly with the molecular or atomic structure. Therefore, all of our results for tooth composition are presented in atomic percentages[25].

For both calcium and phosphorus the mean atomic percentage reduced after demineralization (pH_cycling), then after application of laser there was a slight reduction in mean atomic percentage of both elements. Then after the application of the remineralizing agent (CPP-ACP) there was an increase in the mean atomic percentage of both elements that was further increased when a combination of CPP-ACP and laser was used. A maximum increase of the mean atomic percentage of both elements was recorded when the laser was applied first followed by CPP-ACP agent. For oxygen, results revealed that the mean atomic percentage of oxygen increased in demineralized group as compared to sound group with further slight increased in the lased group. Further progressive reduction was recorded for the atomic percentages of oxygen in the three remaining groups ( CPP-ACP, CPP-ACP followed by laser, Laser followed by CPP-ACP ). Statistical difference was not significant ($p > 0.05$) for phosphorus, but highly significant ($p < 0.01$) for both calcium and oxygen.

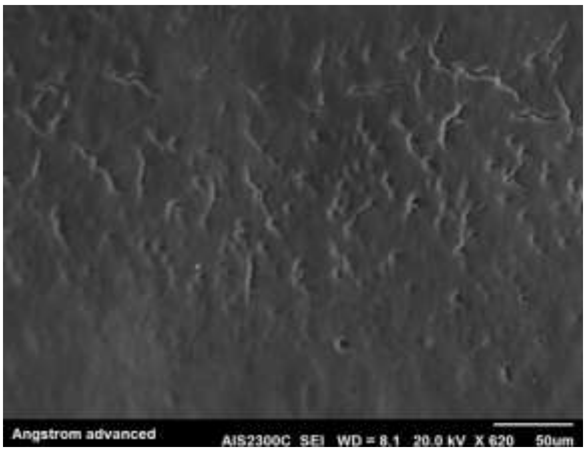
**Table (1) :Mean Values and Standard Deviations of Atomic Percentages for Each Element.**

| Group | | Elements | | |
|---|---|---|---|---|
| | | O | P | Ca |
| Sound | Mean♦ | 63.25 | 14.32 | 22.44 |
| | ±SD | 2.32 | 1.08 | 1.30 |
| pH | Mean♦ | 68.28 | 13.09 | 18.64 |
| | ±SD | 2.05 | 2.59 | 0.59 |
| Laser | Mean♦ | 69.40 | 12.83 | 17.77 |
| | ±SD | 3.66 | 1.07 | 2.60 |
| CPP-ACP | Mean♦ | 66.42 | 13.26 | 20.32 |
| | ±SD | 3.40 | 1.34 | 2.10 |
| CPP-ACP +Laser | Mean♦ | 59.60 | 14.36 | 26.04 |
| | ±SD | 0.87 | 3.08 | 2.62 |
| Laser+ CPP-ACP | Mean♦ | 52.76 | 16.25 | 30.99 |
| | ±SD | 0.37 | 1.65 | 2.02 |
| F | | 25.663** | 1.048 | 16.509** |
| Df | | 5 | 5 | 5 |
| P-value | | 0.000 | 0.405 | 0.000 |

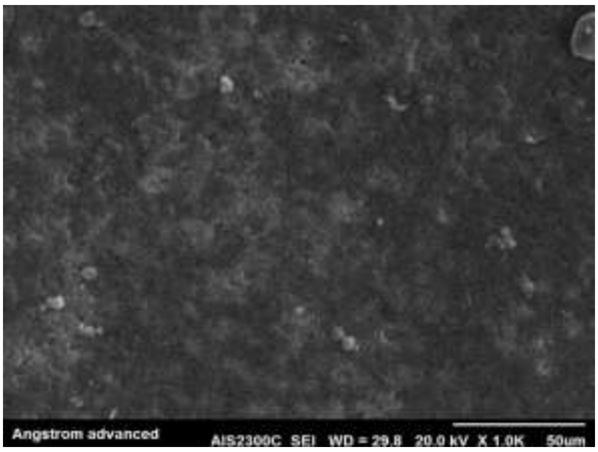
**** Highly significant (p< 0.01)**
**♦ sample size = 3**

**Scanning Electron Microscopy examination:**

The images of scanning electron microscope show the structural change of enamel surface of each group as shown in figure (2). For sound enamel surface Figure (2a) scanning electron microscopic image demonstrating enamel prisms. Enamel surface devoid for any surface deposition or alteration. For Figure (2b) scanning electron microscopic image for enamel surface treated with pH-cycle showing ill defined enamel prisms ,losing their normal architecture with destruction of prismatic sheath and interprismatic substance. Regarding Figure (2c) scanning electron microscopic image of enamel surface treated with CPP-ACP demonstrating deposition of few scattered granules particle of mineral crystals and globule with a crack line seems to be coated with amorphous minerals. Eroded area on enamel surface can be detected. Figure (2d) scanning electron microscopic image of enamel surface irradiated with $CO_2$ laser showing melted area as a holes and fine cracks can be defined. Relatively some smooth area can be seen. While Figure (2e) scanning electron microscopic image of enamel surface treated with a combined application of CPP-ACP followed by $CO_2$ laser demonstrating an area with smooth, homogeneous surface, while others show globular crystals. Presence of few enamel prisms can be detected. Figure (2f) scanning electron microscopic image of enamel surface treated with a combination of $CO_2$ laser followed by application of CPP-ACP shows areas of a coalescing of deposit globules of mineral crystals in a linear manner.




**(a)**

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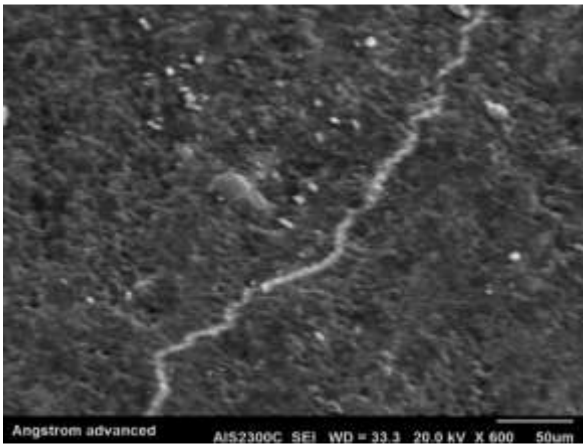
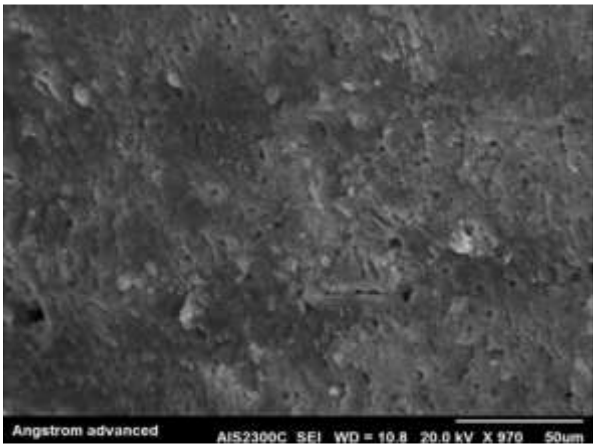
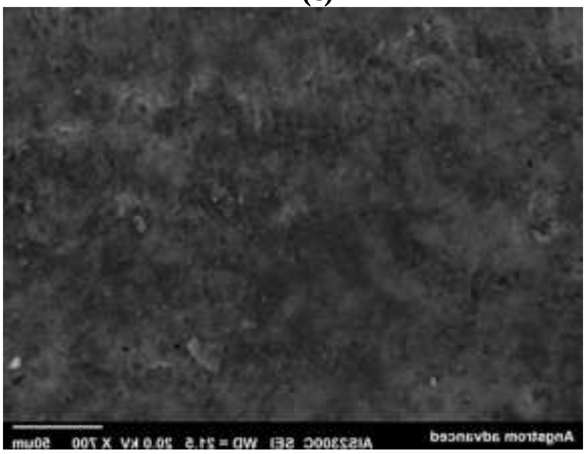


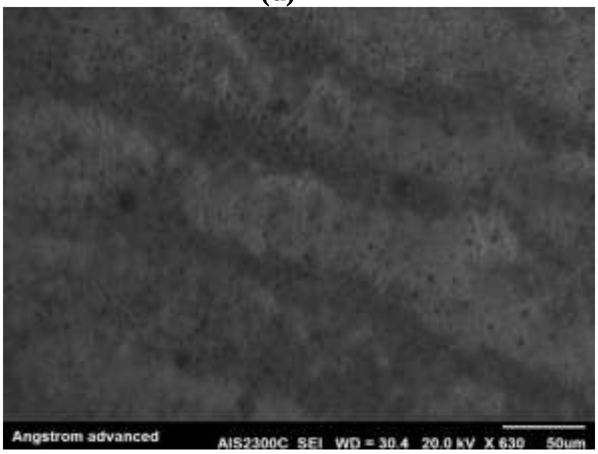

**(c)**




**(d)**



**(e)**




**(f)**

**Figure(1): SEM image (a):surface of sound enamel ,(b): surface of enamel after PH cycling , (c): surface of enamel after treated by CPP-ACP , (d): surface of enamel after treated by $CO_2$ laser of 0.852 watt, (e) surface of enamel after treated by CPP-ACP followed by $CO_2$ laser , (f): surface of enamel after treated by $CO_2$ laser followed by CPP-ACP**.

## Discussion

In the current study data obtained by EDS analysis revealed that both calcium and phosphorous mean atomic percentages reduced after the teeth were subjected to pH_cycling (de- and remineralization sequence) as compared to control ( sound teeth). This is because any reduction in the PH of the surrounding environment below the critical pH that is about 5.5 this will create an acidic media that cause outward movement of tooth minerals (calcium and phosphorous) that constituted enamel hydroxyapatite crystals leaving behind micropores or microspaces[26] and this is confirmed by SEM micrograph for demineralzed enamel surface.

When teeth were irradiated with $CO_2$ laser there was a reduction in both calcium and phosphorous mean atomic percentages as compared with demineralized teeth. This finding was inconsistent with that of Aminabadi et al. (2015)[15] study who found an increase in calcium and phosphate mean weight percentages after lasing as compared to demineralzed group. This is probably because when teeth were irradiated with laser there is an interaction between light and biological constituents of enamel. In case of absorption by dental enamel, the irradiated energy is converted directly into heat[25]. As a result of heat generation melting and re-solidification processes will occur with formation of holes (microspaces) on the enamel surface and this is confirmed by SEM micrograph[27]. This melting process with the formation of microspaces might affect calcium and phosphorous ions level in the outer enamel surface.

After application of CPP-ACP agent (Remineralizing agent) both calcium and phosphorous mean atomic percentages increased as compared with demineralzed and lased groups probably due to deposition calcium and phosphorous ions on enamel surface and subsurface layers, and thereby, formation of hydroxyapatite crystals[28,29]. In addition the presence of CPP prevents rapid transformation of calcium phosphate phase; thus, the ions will be stabilized and maintained in close proximity to enamel lesion thereby decreasing demineralization and enhancing remineralization of enamel lesions[30]. Also, this is further confirmed by SEM micrograph that demonstrated the presence of granules of mineral crystals with the crack line covered with amorphous minerals. Also, this finding was recorded by Aminabadi el at. (2015)[15].

Also results revealed that enamel samples treated with CPP-ACP agent followed by laser showed further increase in calcium and phosphorous mean atomic percentages as compared to the previous four groups. This is possibly due to the synergistic effect of both CPP-ACP agent and laser in remineralizing the initial caries-like lesion as discussed previously. Also, this is confirmed by SEM micrograph that revealed smooth and homogenous surface with globular crystals. The same finding was also recorded by Aminabadi el at. (2015)[15].

Finally, when teeth were irradiated with $CO_2$ laser first, followed by CPP-ACP agent application maximum increase in calcium and phosphorous mean atomic percentages was reached probably because when laser applied first it creates holes in addition to microspaces that would be filled with calcium and phosphorous ions that are provided by CPP-ACP agent[15]. Also, this is supported by SEM micrograph. Nearly the same finding was recorded by Khamaeel (2013)[31] study. In this study, she found an increase in fluoride uptake by enamel surface when laser was applied before the reminaralizing agent (NaF and APF) as compared to the groups in which reminaralizing agents were applied first then irradiation was used.

It is worth to mention that enamel is composed almost exclusively (more than 95 wt%) of hydroxyapatite crystals[32]. However, these inorganic components representing only 87% of the total volume that means 13% of the volume of enamel is composed of organic material (matrix). This organic, protein-rich matrix is mainly water (11% of the total volume of enamel)[33].Therefor, the melting process caused by laser might cause evaporation of water that lead to the formation of holes on the enamel surface (which is confirmed by SEM micrograph).

Also results revealed that the atomic percentages of oxygen increased and decreased in an adverse direction to that of calcium and phosphorous atomic percentages. In fact, this is difficult to be explained because the exact chemical compounds or enamel crystals that would be formed after application of laser and CPP-ACP agent is not exactly known. However, since the EDS analysis was used that express the elements in enamel surface in atomic percentage, therefore the increase in percentage of one element would affect the percentages of other elements in the chemical compounds to achieve balanced chemical compounds and also the total percentage doesn't exceed 100%.

It is recommended that more advanced technologies that revealed crystallographic and chemical changes that occur after application of laser and remineralization agent is advocated to be used in the future studies.

Also, SEM image showed that the surface of enamel treated by $CO_2$ laser first followed by CPP-ACP was smoother than other groups because new hydroxyapatite and fluorapatite crystals are formed on the enamel surface, coating the cracks to form a smoother surface[15].

## Conclusion

The results of this study suggested that the irradiation of $CO_2$ laser at power of 0.852 watt to the enamel before the application of CPP-ACP agent was significant higher than after them or were used with unlased enamel .

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