Effects of Lasers and Fluoride on the Acid Resistance of Human Enamel with Incipient Carious Lesions

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Abstracts

Objectives

In order to preserve the maximum amount of healthy enamel and increase the acid resistance of an incipient carious lesion, CO_2 laser, Nd:YAG laser and APF were used to treat incipient carious lesions, then their effects were compared.

Materials and Methods

One hundred and twenty samples from human caries-free premolars were immersed in pH-cycling solution for 2 days for incipient carious lesion formation. Then all the samples were divided into 4 groups randomly; control group, CO_2 laser group, Nd:YAG laser group and APF group, and the lesions were treated by CO_2 laser, Nd:YAG laser irradiation or APF application respectively. All the samples were immersed in pH-cycling solution a second time for 2 days. As for the acid-resistance evaluation, the calcium concentration dissolved from the enamel surface was analyzed by ISE-trol. Scanning electron microscopy with energy dispersive X-ray analysis system was also used to assay the geographic change and the components of the enamel surface structures.

Results

According to the acid-resistance evaluation, the control group showed a statistically significant ($P<0.05$) higher calcium concentration compared with the APF group and the other two laser groups. Moreover, the APF group showed a statistically significant higher calcium concentration compared with the Nd:YAG laser and CO_2 laser groups ($P<0.05$). However, there was no substantial statistical difference in the two laser groups ($P>0.05$). From the SEM, the melting and re-crystallization surfaces and crater-like holes, 1-20 μm in diameter, were found on the CO_2 laser and Nd:YAG laser treated enamel surfaces. Finally, in the aspect of the EDS, there was no evidence of new components on the enamel surfaces after lasers and APF were applied.

Conclusions

The application of CO_2 laser, Nd:YAG laser and APF on enamel incipient carious lesions increase acid resistance. Furthermore, the effect of laser irradiation is better than that of APF application.

Key words: Incipient carious lesions, Lasers, Fluoride

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Introduction

Lasers appear to be beneficial to anti-caries of sound enamel surfaces. By changing the surface structure and physical properties, including melting and re-crystallization of the enamel hydroxyapatite crystals (1,2), the enamel acid resistance can be improved and the enamel demineralization can also be significantly inhibited (3-7).

The proper usage of fluoride products also has the same anti-caries effect on incipient carious enamel. Fluoride can act as a general chemotherapeutic agent in the oral cavity via three mechanisms: improving acid resistance of the enamel, enhancing remineralization of the incipient lesion, and interfering with micro-organisms by inhibiting bacterial metabolism and enzymatic processes(8).

The advantages of laser irradiation on sound enamel surfaces, as well as the use of topical fluoride, preventing dental caries or reversing incipient caries (9), have been proved. However, there are few studies about the effects of lasers on enamel incipient carious lesions. Therefore, our study aimed to research: (1) Does the acid resistance of an incipient carious lesion increase after laser irradiation? (2) Do any new crystals or components exist in an incipient carious lesion after laser irradiation? In order to survey the effect of lasers and fluoride, the APF application was also used in the study for comparative analysis.

Materials and Methods

Tooth Selection and Sample Preparation

The teeth collected for use in this study were extracted from patients less than 15 years old who received orthodontic treatment in the Pedodontic Department of Chung-Ho Memorial Hospital, Kaohsiung Medical University, Taiwan. The caries-free status of premolars were certified by visual inspection, and then stored in 0.1% thymol solution for usage. After the roots were removed, all the crowns were divided into buccal and lingual portions, totally, one hundred and twenty samples were collected. Teeth samples were coated with acid-resistant varnish (nail polish) for ten minutes, and a window approximately 2 mm square on the outer enamel surfaces was left un-coated for artificial incipient caries lesion formation.

Artificial Incipient Caries Formation and pH-cycling Process

Each tooth-resin block underwent the pH -cycling scheme individually (10-12) for 2 days, 6 hrs in a demineralization solution (10 ml) and 17.5 hrs (10 ml) in a remineralization solution on each day, producing incipient carious lesions. The demineralization solution at 37°C contained 2.2 mmole/L calcium, 2.2 mmole/L phosphate and 50 mmole/L acetate at pH 5. The remineralization solution at 37°C contained 1.5 mmole/L calcium, 0.9 mmole/L phosphate, 150 mmole/L potassium chloride and 20 mmole/L cacodylate at pH 7. The pH-cycling process started with demineralization phase for 6 hours. After that, all the tooth-resin blocks were removed from the solution and rinsed in double-deionized water for 30 mins, followed by a remineralization phase for 17.5 hours. This process continued for two days. After the incipient carious lesion formation, all the tooth-resin blocks were assigned randomly into 4 groups: control group, CO_2 laser group, Nd:YAG laser group and APF group.

Laser Treatment

Nd:YAG laser (LASER-35, Laser Endo Technic Corp., USA) and CO_2 laser (Denta™ K, ESC/Sharplan, U.K.) were used in the experiment. The fixed parameters were set as follows:

(1)Nd:YAG laser: 6 Watts, 50 Hz, 0.8 milliseconds per pulse and the energy density was 83.33 J/cm^2.

(2)CO_2 laser: 2 Watts, continuous wave model and the energy density was 83.33 J/cm^2.

Prior to laser treatment, the energy outputs were calibrated with their own power meters. The distance between the laser tip and the bject was set to be less than 1 mm to maintain a constant spot size.

Fluoride Application

Acidulated phosphate fluoride, APF (1.23% fluoride, pH 3.5, Pascal Company) was applied on the enamel surfaces for fluoride treatment and the exposure time was 4 mins immersed in the remineralization solution. Then, the samples were washed in distilled water for 5 mins to clean the surfaces.

Acid Challenge

After laser and fluoride treatment, all the treated samples were re-immersed in the pH-cycling solution for 2 days to undergo acid challenge for acid-resistance evaluation.

Acid Resistance Evaluation

The acid-resistance of all samples was evaluated by analysis of the calcium concentration dissolved from the decalcified enamel surfaces in the demineralization solution by electrolyte controls (ISE-trol® , AVL 9180, AVL Scientific Corporation). In addition to the initial calibration, respective calibration was also carried out immediately after every ten analyses.

Scanning Electron Microscopy (SEM) Evaluation

All of the fully-dried samples were coated with a thin layer of gold-palladium approximately 5-10 nm in thickness, and observed under scanning electron microscopy (Jeol®, JSM-5300) at different magnifications, 500X and 1000X. We chose three spots in the central area of the enamel carious lesion in individual samples to perform energy dispersive X-ray analysis system (EDS) evaluation.

Statistical Analysis

In the aspect of acid-resistance evaluation with calcium concentration, we used JMP 5.0 software and established an ANOVA model to assess the effectiveness of the three different treatments and their potential interaction.

Then, Tukey-Kramer HSD was used to assess the statistical significance for the comparisons of all pairs. An alpha level of 0.05 was selected to determine the statistical significance of the results.

Results

The calcium concentrations dissolving from the incipient carious lesions in all four groups are shown in Table 1. According to the acid resistance evaluation, the control group showed the highest Ca^{2+} concentration dissolving from the enamel surfaces, followed by the APF group, while the two laser groups showed the lowest Ca^{2+} concentration.

Among these three groups, a statistically significant difference ($P<0.05$) was observed. Yet, there was no substantial statistical difference ($P>0.05$) between the Nd:YAG laser and CO_2 laser groups.

From the structural observations with SEM, the control group showed surface destruction and enamel prism exposure under the remaining enamel surfaces (Fig.1). However, there was no obvious surface destruction in the APF group, but some grains with various sizes on the enamel surface were observed (Fig.2). As for melting and re-crystallization surfaces, crack lines, abruption and holes from 2 to 5 μm in diameter were found in the Nd:YAG laser group (Fig.3). Similar to the Nd:YAG laser group, the SEM pictures in the CO_2 laser group showed the following phenomenon: (1) melting, re-crystallization and glaze-like surfaces, (2) crater-like holes with various diameters, (3) few crystal grains in the CO_2 laser treated group (Fig.4). Comparing the two laser groups, it seemed that the crater-like holes existing in the CO_2 laser group were shallower than those in the Nd:YAG laser group. The melting and re-crystallization surface in the CO_2 laser group was more glazed than that in the Nd:YAG laser group.

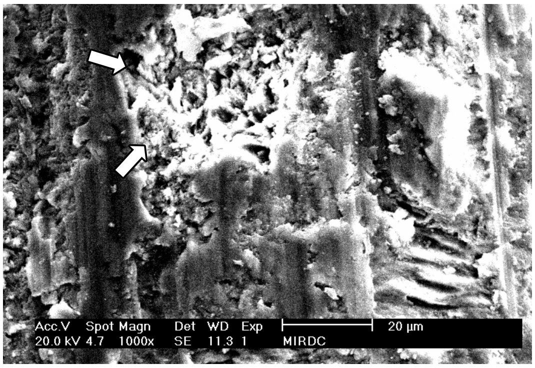

Fig. 1

SEM of control group showed surface destruction and enamel prism exposure (arrow) (magnification 1000×)

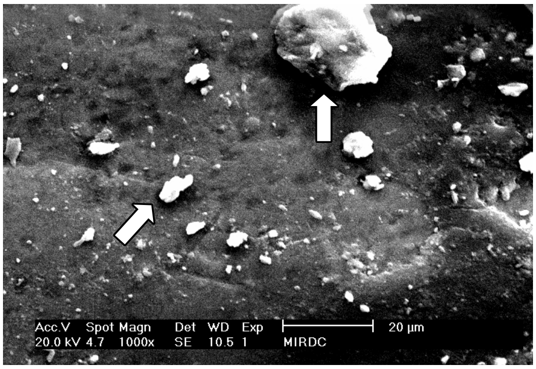

Fig. 2

SEM of APF group showed no evident surface destruction but some grains with various sizes on the enamel surface (arrow) (magnification 1000×)

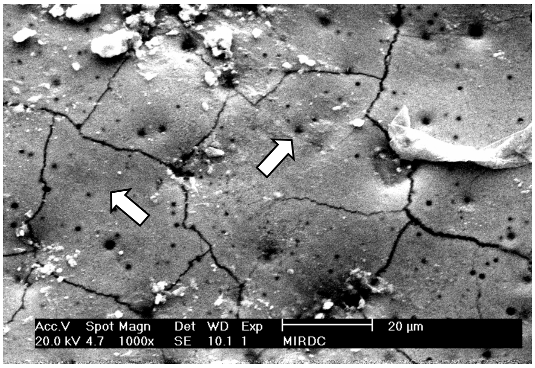

Fig. 3

SEM of Nd:YAG laser group showed melting and re-crystallization surfaces, crack lines, abruption and holes from 2 to 5 µm in diameter (arrow) (magnification 1000×)

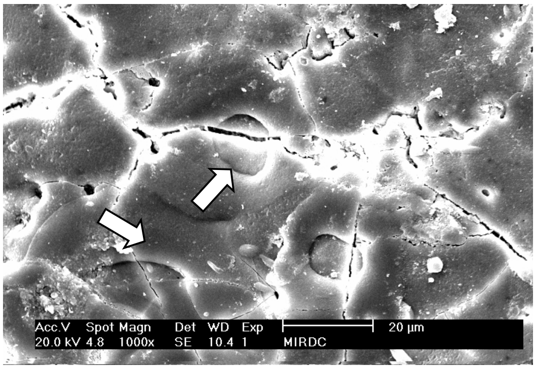

Fig. 4

SEM of CO_2 laser group showed melting, re-crystallization and glaze-like surfaces, and crater-like shallow holes (arrow) (magnification 1000×)

Discussions

It is well-known that caries progression is a dynamic process and involves two major procedures: (1) the dissolution of the enamel hydroxyapatite crystals and (2) the diffusion of the ions, such as calcium, phosphate, and hydrogen ions, into and out of the enamel surfaces(13). The first sites of acid attack are microstructures like inter-crystalline space and inter-prismatic space and some development defects especially the holes and cracks on enamel surfaces(14). When an incipient carious lesion formats, it consists of a sub-surface area of demineralization with an overlying well-mineralized intact surface zone(15). A surface layer with a higher degree of mineralization is more resistant to the acid challenge due to the different biochemical compositions; consisting of a low water content, a low carbonate content, and a high level of trace elements, such as fluoride and chloride(16).

On the other hand, the SEM of the control group in our study showed that enamel surface destruction and prism exposure coincided with the results of Haikel(16). The incipient carious lesions could be divided into four types under SEM observation: (1) areas of apparently intact enamel, (2) focal holes, (3) a prismatic pattern of destruction and (4) an irregular type of destruction.

From the present study, the measurement of calcium concentration of the enamel surface showed that the APF application on the incipient carious lesions increases the acid-resistance and prevents caries progression. It might be that treating the incipient carious lesions with topical fluoride in low concentration transfers the enamel amorphous calcium phosphate to crystalline hydroxyapatite, “improved crystallinity”. In addition, the negative fluoride ions which are adsorbed to the enamel surfaces substitute the hydroxyl positions in the hydroxyapatite to form new, more acid resistant and larger size crystals, fluoroapatite, during the remineralization process(17). Next, fluoride performed its anti-caries action by accelerating or enhancing remineralization, a normal process that occurs if the enamel surface is intact and if the calcium and phosphate ions are in adequate levels in the saliva. The negative fluoride ions diffused into the subsurface zone and the positively charged calcium ions followed by the phosphate ions are attracted into the remineralizing areas(9,18).

Our SEM observation of the APF group showed no prominent destruction on the enamel surface, which was seen in the control group. The ground-glass-like enamel surface in the APF group was not as glazed as the melting surface in the laser groups. In addition, we also found some various size granules on the surfaces in the APF group. The main products of topical fluoride application on the enamel surfaces were calcium fluoride or fluoride-like substances (19,20) and they were resistant enough to serve as a fluoride reservoir for extended periods of time(21).

Laser irradiations on a sound enamel surface increases enamel acid resistance and inhibits carious lesion formation. The reasons might be summarized as follows: (1) the permeability of ions diffusing from the lased sound enamel surface during the demineralization period decrease due to the laser effects of melting and sealing(22). Generally, the cutting and drilling actions occur only with relatively long interaction times together with high accumulated laser pulse energy (>100 J/cm^2); otherwise, the rapid melting and re-crystallization effects only occur with short interaction times and relatively low pulse energy densities (1-100 J/cm2)(23). (2) The enamel compositions change to low solubility to acid after laser irradiation. Tetracalcium phosphate, $Ca_4(PO_4)_2O$, with higher Ca/P ratio and more acid-resistance than hydroxyapatite, were found in the lased sound enamel surface(3). According to the X-ray diffraction, infrared spectroscopy and selected-area electron diffraction, the mineral compositions in the lased sound enamel surfaces were changed to either pyrophosphate(24), tetracalcium phosphate(3,24) or α - and β --tricalcium phosphate(24). (3) The enamel organic matrix composition is influenced by laser treatment(25) and the acid-solubility of enamel is reduced with a decreased amount of carbon in the enamel(26). The enamel with an incipient carious lesion in our study showed that the calcium concentrations dissolved from the lased enamel surfaces were significantly lower than the control group. The results we found indicated that the acid-resistance of the incipient carious lesions increased after laser treatment. The outermost surface of the incipient carious lesion was thought to be more acid-resistant than that of the sound enamel. It might be because the calcium and phosphate that diffused outward from the subsurface precipitated in the outer surface layer of the enamel in the form of a more stable calcium phosphate phase during the decalcification process, thus preserving the surface layer(27). The incipient carious lesions had to progress to a depth of 300 to 500 μm to be clinically detectable and the greatest amount of demineralization occurred 10 to 15 μm beneath the enamel surface. Nelson et al.(3) pointed out that the "surface melting zone" of a lased enamel surface containing crystal melting and re-crystallization is about 5 μm, and the "denature zone", beneath the surface melting zone, in which the organic content changed due to heat treatment of laser is 10 to 20 μm. From the results mentioned above, the effect of laser irradiation is deep enough to influence the central demineralization area of the incipient carious lesion. Meanwhile, the SEM observations in our study showed that the physical changes like melting and sealing of the enamel surface structures and chemical alterations including inner composition and organic matrix caused by the laser high temperature effects still exist in the incipient carious lesions as well as in the sound enamel. Thus, from our study we conclude that using CO_2 laser, Nd:YAG laser or APF on an incipient carious lesion increased acid resistance and inhibited caries progression. The advantages of lasers were greater than APF, but no differences between the CO_2 laser and Nd:YAG laser were found.

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具初期齲齒之牙釉質表面經雷射與氟化物處理後其抗酸性評估

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摘要

目的

為了保留更多的健康牙釉質並增加初期齲齒病灶區的抗酸侵蝕能力，在本實驗中使用CO_2 雷射、 Nd:YAG雷射與氟化物來處理初期齲齒並比較其效果。

材料與方法

120個取自人類無齲齒病灶之小臼齒的牙齒樣本，將其浸泡於酸鹼循環溶液中2天以形成初期齲齒病灶區。隨機將所有樣本分成4組：控制組、CO_2 雷射組、Nd:YAG雷射組與APF組；各組別再依據該組條件進行雷射或氟化物的處理。接下來將所有牙齒樣本再次浸泡入酸鹼循環溶液中2天進行酸侵蝕，利用ISE-trol®分析溶液中由牙釉質表面溶解出來的鈣離子，以評估各組之抗酸侵蝕能力的差異。同時利用掃瞄式電子顯微鏡與x光繞射來分析牙釉質表面型態的改變與組成結構的成分。

結果

在鈣離子溶出分析以評估抗酸侵蝕能力方面，控制組較其他組別有較高的鈣離子溶出濃度並在統計上有顯著差異($P<0.05$)。此外APF組較兩雷射組有較高的鈣離子溶出濃度，在統計學上亦呈現出顯著差異($P<0.05$)，但在CO_2 雷射組與 Nd:YAG雷射組間則無顯著差異出現($P>0.05$)。從掃瞄式電子顯微鏡上觀察可見在CO_2 雷射組與 Nd:YAG雷射組的牙釉質表面，出現

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熔融再結晶的現象及直徑1-20 μm類似火山口狀的坑洞。最後經由X光繞射分析，無論是雷射組別或是APF組別的牙釉質表面皆無發現新的組成成分。

結論

在具有初期齲齒的牙釉質表面使用CO_2雷射、Nd:YAG雷射及APF皆可增加其抗酸侵蝕能力，就效果而言雷射較APF有更佳的表現。