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Influence of CO2 Laser Irradiation and CPP-ACP Paste Application on Demineralized Enamel Microhardness

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Abstract

Introduction: It has been suggested that the application of casein phosphopeptide-amorphous calcium phosphate paste (CPP-ACP) and CO2 laser irradiation on enamel could increase the resistance of enamel to caries and acid attacks. The aim of the current study was to compare the influence of CPP-ACP paste application and irradiation of CO2 laser on microhardness of demineralized enamel.

Methods: Thirty sound maxillary extracted premolars were selected. The crowns were cut at the cervical line and were split into facial and palatal halves. Specimens were mounted in self-cure acrylic blocks in such way that the enamel surface was exposed to 4×4 mm. After a pH cycling of the specimens, they were randomly divided into 4 groups (n = 15), as follows: CG: Control group, LAS: CO2 laser, CP: CPP-ACP and LASCP: laser combined CPP-ACP treatment. The Vickers microhardness of the specimens was measured (500 g load, 5 seconds, 3 points). Data were analyzed using one-way ANOVA and post hoc Tukey tests ($\alpha = 0.05$).

Results: The lowest mean Vickers microhardness value was observed in CG group (192.57±50.87 kg/mm²) and the highest in LASCP group (361.86±22.22 kg/mm²). There were significant differences between groups ($P < 0.001$). The pairwise comparison of the groups revealed that there were significant differences between these groups: CG versus LAS, CP, LASCP ($P < 0.05$) and LASCP versus LAS and CP ($P < 0.05$). No significant difference between LAS group versus CP group ($P > 0.05$) was observed.

Conclusion: The results of the current study revealed that CO2 laser and CCP-ACP were effective for improvement of enamel hardness value after demineralization. Incorporation of CO2 laser irradiation and CCP-ACP paste application provides additional remineralizing potential for demineralized enamel.

Keywords: CO2 laser; Casein phosphopeptide-amorphous calcium phosphate nano complex; Enamel microhardness.

Introduction

Dental caries have a dynamic process. They initiate in the form of primary subclinical lesions and result in cavity formation following the primary clinical phase.[1,2] In the management of tooth caries, detection of primary lesions and use of remineralizing agents are necessary to prevent caries progression and stop the demineralization process prior to cavitation.[3] It has been confirmed that white spot lesions can be reversed by the use of mineralizing agents with high calcium concentration and that their progression towards cavitation may be prevented.[4-6]

Recently, application of casein phosphopeptide-amorphous calcium phosphate paste (CPP-ACP) has been introduced to increase enamel resistance to caries.[7-9] CPP consist of a cluster of phosphoryl residues that stabilizes ACP nanoclusters in metastable solution. It is a sticky protein that bonds to phosphate and calcium ions and stabilizes them in an amorphous form.[8] CPP-ACP easily bonds to pellicle, soft tissue plaque and even enamel hydroxyapatite. It can also be incorporated into toothpastes and mouth rinses for more efficient caries prevention.[10]

CPP-ACP nano complex has shown cariostatic properties in in-vitro, in-situ, animal models and human studies.[8-13]

On the other hand, laser technology has become highly popular in dentistry and medicine in recent years.[14] Many

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studies have evaluated the effect of lasers on the enamel and dentin.[15-17]

Among all of dental lasers, $CO2$ laser has the maximum absorption rate in hydroxyapatite and operates at a wavelength of 9-11 µm.[18,19] This district of wavelength band coincides closely with some of the apatite absorption wavelengths.[20] Therefore, Co2 laser irradiation causes chemical and morphological changes of enamel that subsequently alters the mineral and organic content of the tooth structure and increases enamel resistance to acid attacks.[19-21] In addition, when considering dental caries CO2 laser irradiation improves the efficacy of fluorides.[18,22,23] Irradiation of CO2 laser with fluoride application may produce fluorohydroxyapatite and calcium fluoride (CaF2) on the enamel surface. During episodes of enamel demineralization, these compositions serve as a depository of fluoride and are used up later for remineralization process.[20] Nonetheless, the efficacy of lasers and remineralizing agent for promoting remineralization of enamel has not been investigated sufficiently.[24,25]

The current study evaluated the effectiveness of CO2 laser, in association with CPP-ACP paste, on Vickers microhardness of demineralized enamel.

Methods

Thirty intact human maxillary premolars extracted during the previous 3 months were collected from dental centers and private office. The teeth were cleaned using a scalpel and toothbrush and immersed in 0.5% chloramine solution. They were examined under a stereomicroscope (Olympus, Shinjuku, Tokyo, Japan); to ensure the absence of structural defects, cracks or caries. The crown of each tooth was removed at the cervical line by using a diamond saw (Microslice 2, Metal Research, Cambridge, UK) with the water-coolant stream. Subsequently, the crowns of the teeth were sectioned into facial and palatal parts. Specimens were mounted in autopolymerizing acrylic resin blocks (Acropars, Kaveh, Tehran, Iran) in such way that a 4×4 mm facial or palatal enamel surface was exposed. In order to prevent dehydration of the teeth, they were stored in distilled water. The teeth were immersed in demineralization solution (pH = 4.6) for 8 hours and then removed and immersed in artificial saliva solution for a period of 1 hour. Afterwards, the teeth were immersed in a remineralizing solution (pH = 7) for a period of 15 hours. This cycling continued for 14 days to demineralize the enamel surface. The demineralizing and remineralizing solutions were refreshed every two days.[26] The demineralizing solution comprised of 0.05 mM $CaCl_2$, 2.2 mM NaH_2PO_4 and 50 mM acetic acid. The solution containing 20 mM HEPES, 1.5 mM Ca^{2+} ($CaCl_2$), 0.9 mM phosphate (KH_2PO_4) and 1 ppm fluoride (NaF) was used as a remineralizing solution. Mounted specimens were randomly divided into four groups of fifteen (n = 15), according to the treatments:

CG: Specimens received no treatment (control group). LAS: Specimens received CO2 laser irradiation.

CP: Specimens were treated with CPP-ACP. LASCP: Specimens were treated with CPP-ACP, then received CO2 laser irradiation.

Application of CPP-ACP

In CP and LASCP, according to the manufacturer's instructions, CPP-ACP paste (MI Paste, Recaldent™, GC Co., USA) was applied directly on the demineralized enamel surface for five minutes and then washed.[11,26]

CO2 Laser Irradiation

In LAS and LASCP, the surface of the specimens was lased with a CO2 laser at a wavelength of 10.6 µm (SmartXide2; DEKA Laser, Florence, Italy). Laser parameters were adjusted as follows: Power of 0.7 W, pulse frequency of 50 Hz, focal spot of 0.2 mm, pulse duration of 0.4 milliseconds, non-contact mode with 10 mm distance from the hollow tube tip to the tooth surface, spot size of 0.4 mm. Average power output was measured by using a power meter (Model 37-3002, Scientech Inc, Colorado, USA) and determined at 0.68 W. Thus, the power density was 5.41 W/mm^2 and the laser fluency applied to enamel was 10.66 J/cm^2. The demineralized enamel surface was scanned by the laser hand piece with a circular motion starting from the center and moving outward with a 2 mm/s speed for 5 seconds simultaneously with air spray (60%) application in a circle with 3 mm diameter[27] and total energy of 3.5 J.

Microhardness Testing

Vickers hardness test was performed for all treated specimens after storage in distilled water for 48 hours. Values were recorded with a microhardness tester microscope (Micrometer 1, Buehler, Lake Bluff, IL, USA) using 500-g load for 5 seconds in 3 points.[28] The mean of the microhardness values for points was considered for the micro-hardness value of each sample.

SPSS software (version 18) was used to categorize the data. Data were analyzed by one-way ANOVA and post hoc Tukey tests at the level of $\alpha = 0.05$ significance.

Results

Means and standard deviations are presented in Table 1. The lowest mean Vickers hardness value was observed in CG (192.57 ± 50.87 kg/mm^2) and the highest in LASCP (361.86 ± 22.22 kg/mm^2).

One-way ANOVA indicated that there were significant mean differences between groups ($P < 0.001$). The pairwise comparison of groups revealed that there were significant differences between these groups: CG versus LAS, CP, LASCP ($P < 0.05$) and LASCP versus LAS and CP ($P < 0.05$). No significant difference between LAS group versus CP group ($P > 0.05$) was observed (Table 1).

Table 1. Comparisons of the Microhardness Mean Values (kg/mm[2]) in the Groups Studied

Groups	Mean ± SD	95% CI Form the Mean		*P* Value[a]
		Lower Bound	Upper Bound	
CG (n=15)	192.57 ± 50.87 [a]	5.8286	7.5644	0.0001
LAS (n=15)	298.60 ± 25.30 [b]	8.3897	9.5903	
CP (n=15)	302.22 ± 23.81 [b]	6.5440	7.8583	
LASCP (n=15)	361.86 ± 22.22 [c]	6.7401	7.9352	

Abbreviation: SD, standard deviation.

[a] One-way ANOVA.

Values with different uppercase letters show significant difference according to Tukey test ($P<0.05$).

Discussion

In contemporary dentistry, prevention of caries is superior to treatment.[29] Conventional tooth treatment eliminates the carious tissue and restores cavity with restorative materials. Non-invasive approach for carious lesions is now considered by many researchers.[30]

This study evaluated the effect of CO_2 laser irradiation and CPP-ACP paste application on enamel remineralization of demineralized enamel. The results of the current study showed that CO_2 laser with a 10.6 µm wavelength and controlled parameters significantly increased enamel surface microhardness ($P<0.05$). Previous studies confirm these results.[20,23,27] Some studies have reported that lasers with an emission wavelength of 10.6 µm are more efficient for caries prevention.[27,31,32] Laser irradiation changes the ratio of calcium to phosphate and decreases the carbonate/phosphate ratio in tooth structure.[33] The loss of carbonate results in more stable and less acid soluble products and increases enamel caries resistance. The decrease in carbonate content caused by CO_2 laser irradiation was confirmed in previous studies.[34,35] The enamel carbonate content can be reduced or eliminated depending on the amount of energy applied, and crystallinity can be increased.[36] Laser causes re-crystallization in the enamel structure and makes enamel more resistant to acid dissolution.[21] Correa-Afonso et al reported that the presence of carbonate generates unstable apatite crystals.[37,38] It was reported that CO_2 laser could increase the crystalline stability making the enamel less vulnerable to acid, and increase fluoride uptake by the enamel.[39]

A SEM assessment indicated CO_2 irradiation on human enamel forms melted irregular masses of hydroxyapatite. These masses had coalescence appearance and it seems that recrystallization occurred.[21]

Chiang et al reported that change in enamel matrix permeability occurs after lasing. It produces a decrease in acid diffusion, and alterations in organic and inorganic components of the enamel lead to increased enamel microhardness and decreased enamel susceptibility to acid.[40] Also, Daniel et al showed that CO_2 laser beam was effective in the reduction of mineral loss around composite resin filling on root surfaces and CO_2 laser could be a resource in the prevention of secondary caries lesions on elderly population.[33]

In the current study, CPP-ACP was used alone and in conjunction with laser to treat demineralized enamel lesions. The results showed that CPP-ACP paste significantly increased the microhardness of demineralized enamel. It has been reported that a stable complex formed by casein phosphopeptide-amorphous calcium phosphate can be used for remineralization of primary enamel lesions.[41] CPP-ACP maintains a saturated state of phosphate and calcium on the enamel surface, and by doing so, decreases demineralization and increases remineralization.[26,42] CPP with the cluster sequence of Ser(p)-Ser(p)-Ser(p)-Glu-Glu can bond to ACP in the solution. This process retains a high level of phosphate and calcium ions on the surface of lesions, prevents demineralization, and improves remineralization of enamel.[7,11]

CPP-ACP paste can bond to enamel, biofilm and soft tissues around the enamel and guides the free calcium and phosphate ions into enamel crystals, resulting in the reformation of apatite crystals.[43]

In other words, CPP-ACP enhances the remineralization of carious lesions via saturation of enamel minerals.[7,38] Previous studies have also demonstrated the role of CPP-ACP in preventing the process of demineralization. Our study findings are in agreement with these studies.[11,26,44]

In the current study, no significant difference was noted between the impact of laser treatment and application of CPP-ACP paste on Vickers microhardness improvement of demineralized enamel. In other words, both methods equally enhanced the process of remineralization of lesions. Also, it was noted that CPP-ACP paste in conjunction with laser significantly increased the microhardness of enamel compared to the value in groups treated with laser or CPP-ACP paste alone (Table 1). CPP delivers small, stable hydrated calcium phosphate clusters into the intercellular matrix and localizes calcium and phosphate ions for easy access. These tiny clusters act as a reservoir and are refilled with calcium and phosphate ions.[8] By doing so, they enhance the effect of laser on inhibiting demineralization and increasing remineralization.

This result confirms findings of Niazy's study regarding the synergistic effect of CO_2 laser in conjunction with CPP-ACP paste on the enhancement of remineralization.[24] In fact, the change caused by laser enhances the penetration of CCP-ACP nanocomplex into HA crystals in deeper layers.[40,45] Thus, it has higher efficacy than the application of laser or CPP-ACP alone for remineralization.

Similar studies are required to compare other preventive measures, such as the use of fluoride and xylitol gums. This study had an in-vitro design and future studies are required to assess the efficacy of these techniques in a clinical setting.

Conclusion

Within the limitations of this study, our results demonstrated that CO2 laser irradiation, at 10.6 μm and application of CPP-ACP increased the microhardness of demineralized enamel. Incorporation of the CO2 laser to CCP-ACP formulation provides additional remineralizing potential.

Conflict of Interests

All of the named authors had no conflict of interest.

Ethical Considerations

The protocol of this investigation was approved by the Ethics Committee of Hamadan University of Medical Sciences.

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