Preventing enamel caries using carbon dioxide laser and silver diamine fluoride

Abstract

***Objectives**: To investigate the caries prevention effect of carbon dioxide (CO_2) laser (λ = 10,600 nm) irradiation followed by the application of silver diamine fluoride (SDF) on enamel.*

***Materials and methods**: Forty human enamel specimens were randomly allocated to four groups. Group-1, specimens were treated with SDF. Group-2, specimens were irradiated with a CO_2 laser. Group-3, specimens were irradiated with a CO_2 laser then treated with SDF. Group-4, specimens had no treatment. All specimens were subjected to pH-cycling for cariogenic challenge. Lesion depth, micro-hardness, surface morphology and elemental analysis were assessed.*

***Results:** The lesion depths for Groups 1 to 4 were 33±16 µm, 80±9 µm, 18±15 µm and 102±9 µm, respectively ($p < 0.001$; Group-3 < Group-1 < Group-2 < Group-4). Knoop hardness values for Groups 1 to 4 were 61±19, 68±20, 78±27 and 36±8, respectively ($p = 0.002$; Group-4 < Groups 1, 2 and 3). The enamel in Group-4 but not in the other groups showed a roughened surface resembling an acid-etched pattern. Calcium-to-phosphorus molar ratios of Groups 1 to 4 were 1.68±60.09, 1.61±0.06, 1.69±0.10 and 1.49±0.10, respectively ($p < 0.001$; Group-4 < Groups 1, 2 and 3).*

***Conclusions**: Separately using the CO_2 laser or SDF enhanced the resistance of enamel against cariogenic challenge. Moreover, there was an additional effect of combined use of the CO_2 laser and SDF for preventing enamel demineralisation.*

***Clinical relevance**: The results can provide useful information to dentists in the use of laser and fluoride for preventing enamel caries.*

***Keywords**: silver diamine fluoride, carbon dioxide laser, caries prevention, enamel*

1 Introduction

Dental caries has been considered one of the oldest and most prevalent multifactorial diseases worldwide. Although continuous efforts have been made to reduce its prevalence, it is still a major oral problem affecting an extremely large majority of adult people and more than half of schoolchildren [1], especially in lower socioeconomic areas. Traditional strategies to treat caries adopted a surgical model which would remove the decay and create a cavity. Technological advances in dentistry have allowed for a shift from the surgical model to a medical model for disease management, and new caries managements emphasise caries prevention and conservation of the tooth structure [2]. Noninvasive methods for caries prevention include oral hygiene, pit-and-fissure sealants, xylitol use and fluoride application. Pit-and-fissure sealants basically work by generating a physical barrier to prevent the access of dental plaque and its acid products from damaging the enamel surface. However, dental sealants are not effective on the smooth surfaces of teeth. Fluorides prevent caries formation by enhancing remineralisation of tooth surfaces at an early stage of mineral loss and making the remineralised surface more resistant to acid attack. Nevertheless, the antimicrobial effect of fluoride is insufficient to prevent caries development.

A recent literature review concluded that silver diamine fluoride (SDF) is a bactericidal agent and can reduce the growth of cariogenic biofilms [3]. SDF, which is a colourless alkaline solution, contains silver and fluoride ions. Silver compounds have been used in medicine and dentistry for a long time due to their antimicrobial properties [3]. Hence, the combined effects of fluoride and silver ions have been hypothesised to prevent formation of new caries and halt caries progression simultaneously [4]. SDF has been used for caries management in Australia, Argentina, Brazil, China and Japan for many years. Currently, it has been approved for clinical use by the United States Food and Drug Administration. Clinical trials reported that SDF was effective in not only preventing enamel caries in the erupting permanent molars [5] and root caries in elderly people [6] but also arresting caries in the primary teeth in children [7]. Laboratory studies showed that SDF had intense antibacterial effects against mono-species [8], dual-species [9] and multi-species [10] cariogenic biofilms. SDF has also been suggested as a preventive method for the development of secondary root caries around glass ionomer cement restorations [11].

Laser therapy has been used as an alternative method of modifying the enamel surface and increasing its resistance to cariogenic challenge since the first ruby laser was developed by Maiman in 1960 [12]. The CO_2 laser appears to be one of the most efficient lasers in caries prevention among the currently available laser systems [13]. The absorption bands of dental hard tissues, which are in the infrared region because of the crystalline hydroxyapatite in the enamel, can efficiently absorb the irradiation from CO_2 lasers [13]. Several studies have demonstrated that CO_2 lasers may be useful for altering the surface morphology and the chemical composition of enamel to inhibit demineralisation both in vitro and in situ [14-16]. Laboratory studies [17,18] also found that there may be a promising result for preventing the demineralisation of enamel when using CO_2 laser irradiation associated with application of titanium tetrafluoride or sodium fluoride. However, some studies [19,20] showed varied results when combining the use of CO_2 lasers and acidulated phosphate fluoride or amine fluoride. Our previous literature review found no study investigated the effects of CO_2 lasers associated with SDF on preventing enamel demineralisation [21]. Thus, the aim of this *in vitro* study was to investigate whether irradiation of enamel with a 10,600 nm CO_2 laser followed by the application of SDF could prevent enamel demineralisation.

2 Materials and Methods

2.1 Specimen preparation

Ten sound human third molars were collected with patients' consent under a protocol approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (IRB UW17-319). Teeth were stored in a 0.1% thymol solution at 4 °C before use. Two-millimetre thick enamel slices were prepared and sectioned into four specimens for four different groups (n = 10 per group). Specimens were polished with micro-fine 4,000 grit silicon carbide paper. A stereomicroscope was used to exclude specimens with cracks or other defects. All specimens were half-covered with an acid-resistant nail varnish (Clarins, Paris, France).

2.2 Experimental treatments

The four enamel specimens from each slice were randomly allocated to four different treatment groups. Group 1 (SDF) – Specimens received a single application of SDF. Group 2 (laser) – Specimens were irradiated with a CO_2 laser. Group 3 (laser + SDF) – Specimens were irradiated with a CO_2 laser then treated with a single application of SDF. Group 4 – Specimens received no treatment (negative control). The SDF used in this study was a 38% w/w solution (Saforide; Toyo Seiyaku Kasei Co. Ltd., Osaka, Japan). A CO_2 laser (λ = 10,600 nm; Opelaser Pro, Yoshida, Tokyo, Japan) was used in the present study. For laser groups 2 and 3, 0.5W continuous wave was selected on laser panel. The mean output power of 0.36W was measured by power meter. The samples were irradiated by hand scanning motion at 5mm per second after a thin layer of water was applied over the surface. Two passes were performed in vertical and horizontal directions to ensure full coverage of the sample. Water layer was applied before each pass of irradiation._The irradiation conditions are summarised in Table 1. The parameters and irradiation technique were selected in our pilot study (data not shown).

2.3 pH cycling

Treated specimens were stored at 25 °C for 30 min before pH cycling. The protocol of pH cycling proposed by Zhao et al. [22] was employed to mimic high caries risk conditions. All specimens were immersed in a demineralisation solution (50 mM acetate, 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4) at pH 4.5 for 16 h followed by an 8 h immersion in a remineralisation solution (150 mM KCl, 20 mM 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid [HEPES], 0.9 mM KH_2PO_4, 1.5 mM $CaCl_2$) at pH 7.0. The pH cycling procedure was performed at 25 °C for 8 days. Solutions were freshly prepared before use.

2.4 Assessment of lesion depth

Specimens (n = 10, per group) were scanned to measure the lesion depth with SkyScan 1076 micro-computed tomography (micro-CT). Specimens were scanned with a spatial resolution of 8 µm at a voltage of 80 kV and a current of 100 µA. The scanning results of each specimen were reconstructed by NRecon reconstruction software (SkyScan, Antwerp, Belgium). Images of specimens were analysed with CTAn software (SkyScan, Antwerp, Belgium) after reconstruction. Cross-sectional images displaying the lesion area of each specimen were located from the reconstructed three-dimensional images [23]. Ten images were randomly selected from those lesion images [24]. The lesion depths were assessed using image analysis software (Image J; National Institutes of Health, USA).

2.5 Micro-hardness test

The surface micro-hardness of the enamel specimens was tested by a Knoop Hardness Tester (Leitz, Micro-hardness Tester; Ernst Leitz Wetzlar GmbH, Wetzlar, Germany) after the measurements of lesion depth. The specimens were placed under the Knoop indenter of the tester. Twenty indentations were made on the lesion surface side of each enamel specimen with a load of 50 gf (0.49 N) for 10 s at each test point [25]. The indentations were approximately 100 μm from each other. The mean Knoop hardness number (KHN) was calculated for analysis. The low KHN value represents a low value in micro-hardness [25].

2.6 Elemental analysis and surface morphology

The enamel specimens were ultrasonically washed in distilled water three times. They were then fixed in 2.5% glutaraldehyde at 4 °C overnight and dehydrated in an ascending series of ethanol solutions. An elemental analysis was carried out to study the calcium (Ca) and phosphorus (P) content of the enamel lesion surface with energy-dispersive X-ray spectroscopy under a scanning electron microscopy (SEM) (Hitachi S-4800 FEG Scanning Electron Microscope, Hitachi Ltd., Tokyo, Japan) at 5 kV in high-vacuum mode. The elemental analysis was performed by measuring three areas (5×5 μm^2) on the surface in each enamel specimen [26]. Furthermore, the Ca/P molar ratio, a parameter related to the solubility of calcium phosphate compounds, was calculated. Generally, a higher Ca/P molar ratio indicates a lower solubility of calcium phosphate compounds. Afterwards, two enamel specimens from each group were sputter-coated with carbon. The surface morphology was examined under SEM.

2.7 Statistical analysis

The normal distribution of the data was checked by the Shapiro–Wilk test of normality ($p > 0.05$). A one-way ANOVA and post hoc test were used to analyse the lesion depth, KHN and the Ca/P molar ratio among the four treatment groups. The SPSS Statistics – V25.0 software (IBM Corporation, Armonk, USA) was used to analyse the data. The level of statistical significance was set at 0.05.

3 Results

3.1 Lesion depth from micro-CT scan

Typical images of micro-CT of the four groups are shown in Figure 1. The mean (± standard deviation) values of lesion depths are shown in Table 2. Group 3 (laser + SDF) had a significantly lowest lesion depth, followed by Group 1 (SDF), Group 2 (laser) and Group 4 (no treatment) ($p < 0.001$, Group 3 < Group 1 < Group 2 < Group 4).

3.2 Micro-hardness test

The mean (± standard deviation) KHN values of the four groups are displayed in Table 2. Enamel specimens receiving no treatment showed a significantly lower KHN compared to the other three groups ($p = 0.002$), while no significant differences of KHN were found among Groups 1, 2 and 3.

3.3 Elemental analysis and surface morphology

The Ca/P molar ratio from Group 1 to Group 4 was 1.68 ± 0.09, 1.61 ± 0.06, 1.69 ± 0.10 and 1.49 ± 0.10, respectively ($p < 0.001$; group 4 < groups 1, 2 and 3). Tukey HSD test identified that there was no significant difference of the Ca/P molar ratio among Groups 1, 2 and 3, whereas Group 4 had a significantly lower Ca/P molar ratio compared to the other three groups.

There was no visible appearance of carbonization of surface change on enamel surface. Representative SEM images of enamel surface morphology are shown in Figure 1. No obvious evidence of ablation or craters was observed on the enamel surfaces after laser irradiation. Enamel surfaces were relatively smooth in Groups 1 and 3, appearing similar to normal enamel structures. Group 2 presented a slightly melting-like appearance, with minor mineral loss in the area of the enamel rod sheath. Rods were exposed in Group 4, while interrod enamel was largely dissolved and profound mineral loss was found.

4 Discussion

This study was the first to investigate the caries prevention effect of CO_2 laser irradiation in combination with SDF on enamel. CO_2 lasers can produce four different wavelengths: 9,300 nm, 9,600 nm, 10,300 nm and 10,600 nm. The 10,600 nm CO_2 wavelength is the earliest wavelength marketed for soft tissue surgeries in medicine and dentistry. In recent years, the 9,300 nm CO_2 laser (SOLEA, Convergence Dental Inc., USA) has been the only hard and soft tissue marketed. Although 10,600 nm is not suitable for dental hard tissue ablation, caries prevention using this wavelength has been extensively studied as reported from our previous literature review [21]. The optical property of 10,600 nm allows deeper penetration and absorption into enamel (12 μm) than 9,300 nm (2 μm) or 9,600 nm (1μm). This can be an advantage in caries prevention as 10,600 nm showed a deeper depth for removal of carbonate content compared with 9,600 nm [27].

The use of the 10,600 nm CO_2 laser alone in caries prevention has been demonstrated in many studies [18,20]. The mechanism of caries prevention by CO_2 laser irradiation is unclear, but reduction in carbonate content is commonly associated with reduction in caries demineralisation. It has been shown that laser irradiation is effective in reducing enamel surface demineralisation with the observation of surface morphological changes including melting and fusion [28-31]. The melted and fused areas formed a physical barrier to acid attack [32,33]. However, such a physical barrier may not be ideal as demineralisation was found along microfractures (50 μm to 75μm in depth) in melted areas [34]. A reduction in enamel demineralisation can also be found without morphological changes in enamel surface [34-37]. A temperature rise between 300 °C and 400 °C induced by laser irradiation can block the diffusion pathway of calcium ions by melting and swelling the organic matrix, which can reduce the calcium loss from the enamel [38]. This is desirable if laser treatment can reduce carbonate content and decrease the solubility of the enamel and dentin surfaces without damage to tooth structures [39].

Although both continuous wave and pulse mode are standard modes of operations in all 10,600 nm CO_2 lasers, pulsed mode is more commonly cited in literature. Laser irradiation with pulse durations in microseconds and milliseconds has been shown to improve the demineralisation resistance of enamel without surface morphological changes [34-37]. Two studies used the continuous wave mode of CO_2 laser on enamel [19,40]. One study showed melting and cracking on the enamel surface after CO_2 laser irradiation [19]. The other study showed promising caries prevention results [40], but the laser parameters and methods were difficult to reproduce both in vitro and in vivo. In this study, the continuous wave mode was selected with water cooling as a novel idea to reduce surface morphological changes. The 10,600 nm CO_2 laser has an absorption coefficient in water (660 cm^{-1}) similar to that of enamel (825 cm^{-1}) [41]. In our pilot study, we investigated the parameter and technique with and without water layer prior to irradiation. SEM observations showed no surface change on samples with water layer but melting and fusion on samples without water layer. A thin layer of water film over the surface can prevent dehydration of the enamel surface, which may decrease the temperature rise induced by lasers. There is also a reduction in the absorption of surface hydroxyapatite as water can compete as an absorption chromophore. Water cooling as a novel idea was therefore employed in this study.

A study found no difference in carbonate content from one to four overlapping passes [28]. As surface morphology showed melting and fusion in all samples, complete elimination of carbonate content might have been achieved in one pass. Furthermore, we calculated the reported fluence of 5 Jcm^{-2} should be 10 Jcm^{-2} which would correlate to the change in surface morphology observed. Although the fluence is half of our study (19 Jcm^{-2}), the speed of motion was one fifth (1mm/sec) of our speed resulting in higher irradiation dose. Although speed of movement in our study reduced irradiation dose (11.8 Jcm^{-2}), the use of water layer was essential in preserving the surface morphology. We described the use of water as competitive chromophore to hydroxyapatite. The slightly melting-like appearance seen under SEM in Group 2 showed that the power and irradiation method designed was effective in temperature control around and below the melting point of hydroxyapatite. The lesion depth of this group seen in micro-CT was significantly lower compared to that of Group 4. This was consistent with the result of microhardness in which Group 2 showed a significantly higher microhardness than Group 4. The increase in microhardness after laser irradiation was in agreement with previous studies [32,33,36,37]. The Ca/P molar ratio is a parameter related to the solubility of calcium phosphate compounds. A lower Ca/P molar ratio indicates a higher solubility of calcium phosphate compounds. The negative control in this study showed significantly lower Ca/P molar ratios compared to the other three groups, which suggested that laser alone, SDF alone or the combination of laser and SDF reduced the solubility of enamel and increased the resistance of acid attack.

Because fluorapatite can be produced by laser irradiation and sodium fluoride [17], there may be an enhanced effect for preventing enamel demineralisation when laser and SDF are combined in application. In this study, micro-CT showed that the lesion depth in enamel treated by laser and SDF was significantly lower than that when treated with SDF alone or laser alone. This demonstrated an additional increase in resistance against cariogenic challenge by laser and SDF. Studies reported no synergistic effect using CO_2 laser irradiation and an acidic fluoride such as acidulated phosphate fluoride or titanium fluoride [18,20,42,43]. CO_2 lasers in combination with neutral sodium fluoride, however, were reported to show a synergistic effect [17]. As SDF is alkaline, it appears that laser irradiation with non-acidic fluoride is better suited for giving additional effect in caries prevention than the laser with an acidic fluoride [18,20,42,43].

In contrast to some fluoride studies, SDF was applied after laser irradiation in this study. The irradiation heat can remove the carbonate phase from enamel crystals, which results in a reduction in acid dissolution on the enamel by transforming carbonated hydroxyapatite into the more acid resistant hydroxyapatite. Applying fluoride to enamel surfaces at this time can lead to the formation of fluorapatite, which is even less acid soluble [44]. The power and method designed in this study were simple and should be replicable with other 10,600 nm CO_2 lasers. In this study, there was no comparison between continuous wave and pulse mode with SDF. We also did not compare SDF with other types of fluoride. More studies are needed to investigate these limitations.

5 Conclusion

With the limitations of this laboratory study, conclusions were drawn as following: using a CO_2 laser alone or SDF alone can protect enamel against artificial cariogenic challenge. Moreover, there is an additional effect from the combined use of CO_2 laser and SDF on preventing enamel demineralisation.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1 Parameters of carbon dioxide laser used in the study

Wavelength	10,600 nm
Repetition rate	1
Mode of operation	Continuous wave
Duty cycle	100%
Mean output power	360 mW
Pulse energy	360 mJ
Beam diameter and area at the sample	1.55 mm / 0.019 cm^{-2}
Fluence	19 Jcm^{-2}
Movement (speed and no. of passes)	5 mm per sec, 2 passes
Irradiation distance	1 mm
Energy per 1.55 mm spot	112 mJ
Dose/1.55 mm spot over 2 passes 112 x 2 / 0.019	11.8 Jcm^{-2}
Water cooling	Water film on hydrated surface

* Output power was measured by PowerMax Pro 150F HD-50mW-150W fan-cooled sensor and LabMax-Pro SSIM Laser Power Meter.

Table 2 Lesion depth and Knoop hardness number (mean ± SD) of enamel (n = 10)

Group (treatment)	Lesion depth (μm)	Knoop hardness number (KHN)
1 (SDF)	33 ± 16	61 ± 19
2 (Laser)	80 ± 9	68 ± 20
3 (Laser + SDF)	18 ± 15	78 ± 27
4 (No treatment)	102 ± 9	36 ± 8
p value	<0.001	0.002
LSD (Group)	3 < 1 < 2 < 4	4 < 1, 2, 3

Figure 1. Scanning electron micrographs of enamel surface morphology (left) and micro-computed tomography images of artificial caries lesion (right)

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