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# Use of a novel 9.3-μm carbon dioxide laser and silver diamine fluoride: Prevention of enamel demineralisation and inhibition of cariogenic bacteria

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ABSTRACT

*Objective.* To investigate the effects of a 9.3-μm carbon dioxide ($CO_2$) laser and silver diamine fluoride (SDF) on the prevention of enamel demineralisation and inhibition of cariogenic bacteria.

*Methods.* Enamel blocks were applied with Laser (Group-1), SDF (Group-2), Laser + SDF (Group-3) and no treatment (Group-4), and then subjected to an 8-day pH-cycling for cariogenic challenge. Lesion depth and cross-sectional micro-hardness were assessed. Surface morphological and chemical changes were studied using scanning electron microscope (SEM) with energy dispersive spectroscopy (EDS). For the antibacterial activity, treated enamel blocks were incubated with *Streptococcus mutans*. The biofilm morphology, kinetics and viability were assessed by SEM, colony-forming units (CFUs) and confocal laser scanning microscope (CLSM), respectively.

*Results.* Lesion depths (μm) for Group-1 to Group-4 were 88 ± 21, 26 ± 11, 13 ± 9 and 115 ± 25, respectively ($p < 0.001$; Group-2 and Group-3 < Group-1 < Group-4). Group-3 had a significantly higher cross-sectional micro-hardness than the other three groups. EDS determined that Group-4 had the lowest calcium-to-phosphorus molar ratio among the groups ($p < 0.001$). SEM images showed apparent bacteria accumulation on enamel surfaces in Group-4, but not in other groups. Log CFUs for Group-1 to Group-4 were 6.2 ± 0.6, 2.9 ± 0.8, 2.2 ± 1.1 and 7.3 ± 0.3, respectively ($p < 0.001$; Group-2 and Group-3 < Group-1 < Group-4). CLSM images revealed that live bacteria dominated in Group-4, but not in other groups.

*Significance.* The irradiation with a 9.3-μm $CO_2$ laser alone can prevent the demineralisation of enamel and reduce the adhesion of cariogenic bacteria. Moreover, adding SDF can significantly increase the preventive effect and antibacterial ability.



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## 1. Introduction

Dental caries is considered a worldwide pandemic disease that affects all ages from early childhood to the elderly. This disease still has significant prevalence in socioeconomically disadvantaged areas, although the prevalence has been reduced in developed countries during the last decades [1]. Caries is regarded as a preventable disease with well-understood aetiology. Several caries-preventive strategies have been adopted in the clinical practice including pit-and-fissure sealants xylitol use and fluoride application. The most common prophylactic interventions are using fluoride varnishes on enamel surfaces as well as sealing of the pits and fissures of permanent molars [2,3]. Even though these prophylactic strategies are universally considered highly effective in caries prevention, they present some questionable aspects. The effectiveness of sealing procedure could be reduced by treatment failures caused by detachments [4]. The availability of fluoride in the oral cavity is effective in enhancing tooth remineralisation even at low concentration [5]. Nevertheless, antimicrobial capability of fluoride is inadequate to prevent caries formation. These issues might require the development of prophylactic approaches to caries prevention.

Laser irradiation has been considered potentially effective in making enamel resistant to acid dissolution since the studies reported by Stern and Sognnaes using Ruby lasers [6]. Laser radiation is absorbed by the mineral, which alters the chemical composition of enamel crystals to a less soluble form by thermal decomposition of carbonated apatite [7]. Carbon dioxide ($CO_2$) lasers are recommended for managing dental hard tissues among the currently various laser systems existing for dental applications [8]. The usual $CO_2$ laser wavelengths are 9.3, 9.6, 10.3 and 10.6 μm. Among these four wavelengths, 9.3 and 9.6-μm $CO_2$ lasers have a higher absorption coefficient to hydroxyapatite than the conventional 10.6 μm [9]. Additionally, 9.3 and 9.6 μm $CO_2$ lasers can achieve high peak power in short pulses in the microsecond range, whereas the conventional 10.6 μm $CO_2$ laser emits millisecond pulses or continuous wave light with possible harmful side effects to the surrounding tissues [10]. Featherstone et al. reported that short-pulsed $CO_2$ laser irradiation could enhance enamel caries resistance under particular laboratory irradiation conditions [7,11]. Rechmann et al. described that 9.3 μm microsecond short-pulsed $CO_2$ laser irradiation increased enamel caries resistance with or without additional fluoride application [12]. Fluorapatite with less solubility than hydroxyapatite was formed when fluoride was applied after the laser irradiation on the enamel surface [12]. Furthermore, it has been suggested that laser irradiation could enhance fluoride uptake to enamel thus increasing its caries-preventive effect [13].

The application of topical fluorides in dentistry is widely used to prevent caries due to its reduction of demineralisation and promotion of remineralisation on tooth surfaces [14]. Silver diamine fluoride (SDF) is one of these topical fluorides. It is considered as a non-invasive, cost-effective and efficient medical device that can be applied on primary and permanent teeth [15]. Recent systematic reviews have shown that SDF is effective in preventing and arresting root caries in elderly people [16], and has been demonstrated to effectively arrest dentine caries in children [17]. Silver ions released from SDF could inhibit the growth of cariogenic bacteria, both in the planktonic and the biofilm phases of growth [18,19]. SDF has been shown to possess intense antibacterial properties against mono-species, dual-species and multi-species cariogenic biofilms in laboratory models [20].

As far as we are aware, no data in the English language literature is available on the effect of 9.3-μm $CO_2$ lasers combined with SDF in preventing enamel demineralisation and inhibiting cariogenic bacteria. Therefore, this laboratory study aimed to evaluate whether irradiation of enamel with a 9.3-μm $CO_2$ laser followed by applying SDF could prevent enamel demineralisation and inhibit the growth of cariogenic bacteria.

## 2. Materials and methods

### 2.1. Specimen preparation

Twenty-four 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). From our previous and pilot studies,the mean lesion depth of the test group was expected to be 150 μm. We aimed to detect a difference of at least 100 μm. Assuming a common standard deviation of 60 μm and with power at 0.80 and $\alpha$ = 0.05, the sample size was at least ten in each group. Extracted teeth were placed in a 0.1% thymol solution at 4 °C before use. Two-millimetre thick enamel slices from buccal surfaces were prepared and polished with micro-fine 4000 grit silicon carbide paper. The slices were then sectioned into four blocks for four treatment groups (n = 24 per group). Blocks with cracks or other defects were excluded under a stereomicroscope. Ten blocks from each group were half-covered with an acid-resistant nail varnish (Clarins, Paris, France) for the demineralisation test. The remaining blocks (n = 14 per group) were used for the antibacterial assessment.

### 2.2. Experimental treatments

Four enamel blocks from each slice were randomly allocated to four treatment groups. Group-1 (Laser) – blocks were irradiated with a $CO_2$ laser. Group-2 (SDF) – blocks received a topical application of 38% SDF (Saforide; Toyo Seiyaku Kasei Co. Ltd., Osaka, Japan). Group-3 (Laser + SDF) – blocks were irradiated with a $CO_2$ laser, followed by a topical application of 38% SDF. Group-4 – blocks received no treatment (negative control). In our pilot experiment, about 17 ± 2 μL of SDF was delivered in a single application. An air-cooled RF-excited laser prototype operating at a 9.3-μm wavelength (Model DL-500; Access Laser Co, Everett, WA, USA) was used in non-contact mode in this study. Our pilot study found several potentially effective irradiation parameters. The laser irradiation conditions used in this study are summarised in Table 1. The parameter used was the best in eliminating carbonate with the least mineral loss against acid challenge. It also did not produce cracks or substantially increase pulp temperature more than 5.5 °C. The flowchart of the experiment design is shown in Fig. 1.

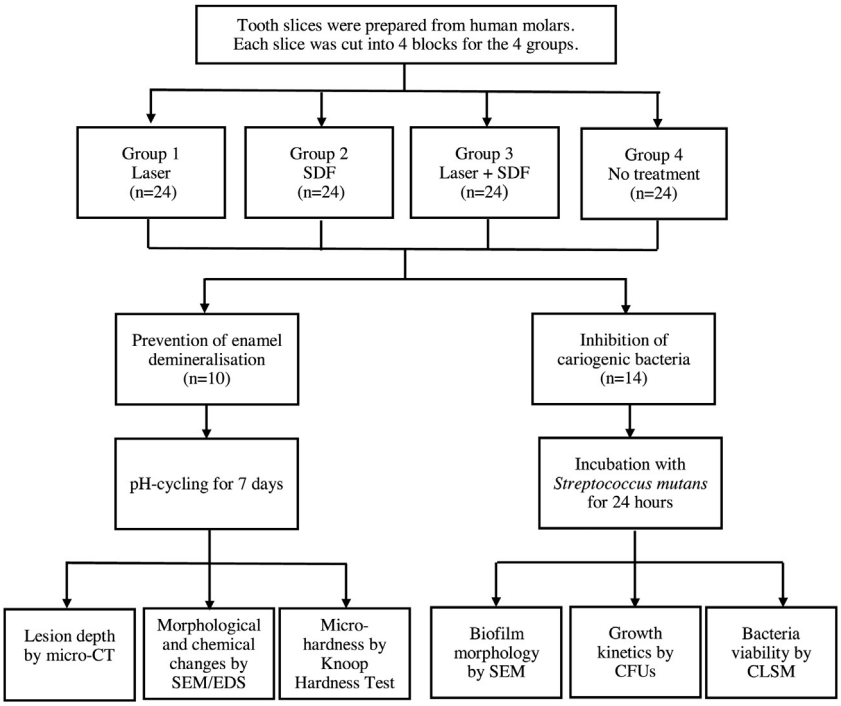


**Fig. 1 – Flow chart of the study.**
**SDF - silver diamine fluoride; Laser - 9.3-μm carbon dioxide laser; SEM - scanning electron microscope; EDS - energy dispersive spectroscopy; CFUs - colony-forming units; CLSM - confocal laser scanning microscope.**

**Table 1 – Irradiation parameters and technique of the carbon dioxide laser.**

| | |
|---|---|
| Wavelength | 9.3 μm |
| Peak power | 534 W |
| Pulse duration | 12.5 μs |
| Pulse energy | 6.68 mJ |
| Frequency | 100 Hz |
| Average power | 0.67 W |
| Beam diameter | 1 mm |
| Spot area | 0.8 $mm^2$ |
| Fluence | 0.85 J $cm^{-2}$ |
| Mode of operation | Pulse mode |
| Duty cycle | 0.13% |
| Motion of irradiation | Fixed spot irradiation |
| 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.

## 2.3. *Assessment of enamel demineralisation*

### 2.3.1. *pH cycling*

The ten half-varnished blocks from each group were subjected to pH cycling for cariogenic challenge using the protocol proposed by Luk et al. [21] to mimic high caries risk conditions. Blocks 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 whole procedure was performed at 25 °C for 8 days. All solutions were freshly made prior to use.

### 2.3.2. *Assessment of lesion depth*

After pH cycling, blocks (n = 10, per group) were examined to measure the lesion depth by using SkyScan 1076 micro-computed tomography (micro-CT). Micro-CT operated at a voltage of 80 kV and a current of 100 μA with a spatial resolution of 8 μm. An NRecon reconstruction software (SkyScan, Antwerp, Belgium) was used to reconstruct the scanning results. After reconstruction, the CTAn software (SkyScan, Antwerp, Belgium) was adopted to view the cross-sectional images showing the lesion area of each block. We randomly selected 10 images from these lesion images for the following assessments [22]. The lesion depths were measured by using image analysis software (Image J; National Institutes of Health, USA).

### 2.3.3. *Micro-hardness test*

After assessing lesion depth, enamel blocks (n = 10, per group) were embedded in acrylic resin and then longitudinally sectioned across the lesion area. Each cut section was flattened and polished. The cross-sectional micro-hardness was determined by means of a Knoop/Vickers Micro Hardness Tester (VH1202, Wilson, Buehler, USA). The block was placed under the Knoop indenter and subjected to a load of 50 gf (0.49 N) for 10 s at each test point [23]. Micro-hardness was examined below the lesion surface of the enamel. Ten indentations were made on each block and the indentations were around 100 μm away from each other. The Knoop hardness number (KHN)

representing the value in micro-hardness was measured for analysis.

#### 2.3.4. Elemental analysis and surface morphology

Ten enamel blocks from each group were studied for elemental analysis and surface morphology. The blocks were ultrasonically washed in distilled water before they were fixed in 2.5% glutaraldehyde at 4 °C overnight. They were then dehydrated in an ascending series of alcohol. The calcium (Ca), phosphorus (P), and fluoride content in lesion surfaces of the enamel were examined for elemental analysis by using energy-dispersive X-ray spectroscopy under a scanning electron microscope (SEM) (Hitachi S-4800 FEG Scanning Electron Microscope, Hitachi Ltd., Tokyo, Japan) at 15 kV in high-vacuum mode. The elemental analysis was carried out by studying three areas ($5 \times 5\ \mu m^2$) on the lesion surface in each enamel block [21]. After elemental analysis, two blocks from each group were sputter-coated with carbon and then observed under SEM for surface morphology. The Ca/P molar ratio relating to the solubility of calcium phosphate was calculated.

### 2.4. Assessment of inhibition of cariogenic bacteria

Fourteen treated blocks from each group were immersed in 1 mL *Streptococcus mutans* (*S. mutans*) bacterial culture with brain heart infusion (BHI) broth and 5% sucrose after sterilisation. They were then incubated anaerobically at 37 °C for 24 h. *S. mutans* ATCC 35668 was commercially obtained from the American Type Culture Collection (ATCC). The bacteria concentration was adjusted to $1 \times 10^7$ CFU/mL.

#### 2.4.1. Biofilm morphology and adhesion

The biofilm morphology was observed under SEM. Enamel blocks with biofilm (n = 2, per group) were fixed in 2.5% glutaraldehyde at 4 °C overnight. Subsequently, they were dehydrated in an ascending series of alcohol, dried in a desiccator and sputter-coated with gold before observation.

#### 2.4.2. Growth kinetics

The growth kinetics of the biofilm was determined by counting colony-forming units (CFUs) of *S. mutans* on the enamel surfaces (n = 10, per group). *S. mutans* from the block surface were resuspended in 1 mL BHI broth. The bacteria suspension was serial ten-fold diluted in sterile BHI broth. Afterward, each dilution was placed in horse blood agar and then incubated anaerobically at 37 °C for 72 h before CFU count.

#### 2.4.3. Bacteria viability

The viability of *S. mutans* in the biofilm was assessed via confocal laser scanning microscope (CLSM). Bacteria on the enamel surface were labelled with two fluorescent probes: propodium iodide and SYTO-9 dye (LIVE/DEAD BacLight Bacterial viability kit, Molecular Probes, Eugene, OR, USA). The live bacteria were labelled in green with the SYTO-9 probe, whereas the dead bacteria were labelled in red with the propodium iodide probe. Images of the labelled biofilm were captured by CLSM (Fluoview FV 1000, Olympus, Tokyo, Japan). Three-dimensional reconstruction was conducted by Imaris 9.3.0 (Bitplane, Zürich, Switzerland).

### 2.5. Statistical analysis

All data were assessed for normal distribution by the Shapiro–Wilk test ($p > 0.05$). One-way ANOVA with Bonferroni test was used to analyze lesion depth, micro-hardness, Ca/P molar ratio, fluoride weight percentage, and the CFUs of the four groups. All data were analysed with IBM SPSS V20.0 software (IBM Corporation, Armonk, NY, USA) and the level of significance was set at 5%.

## 3. Results

### 3.1. Lesion depth from micro-CT scan

Representative images from micro-CT of the four groups are displayed in Fig. 2. The mean (±standard deviation) values of four groups of lesion depths are shown in Table 2. Group-4 (No treatment) had a significantly higher lesion depth than the other three groups, whereas no significant difference was found between Group-2 (SDF) and Group-3 (Laser + SDF) ($p < 0.001$, Group-2, Group-3 < Group-1 < Group-4).

### 3.2. Micro-hardness test

The mean (±standard deviation) KHN values of the four groups are shown in Table 2. The Group-4 (No treatment) had significantly lower KHN values compared with other three groups ($p < 0.001$). Enamel blocks receiving laser combined with SDF application showed a significantly higher KHN than those treated with the laser alone or with SDF alone ($p < 0.001$).

### 3.3. Elemental analysis and surface morphology

The Ca/P molar ratio and fluoride weight percentage for the four groups are shown in Table 2. Multiple comparisons revealed that Group-4 (No treatment) had a significantly lower Ca/P molar ratio than other three groups ($p < 0.001$), while no significant difference was found among Groups 1, 2 and 3. Group-2 and Group-3 had a significantly higher fluoride weight percentage than Group-1 and Group-4 ($p < 0.001$). No significant difference was found between Group-2 and Group-3, as well as Group-1 and Group-4.

Typical SEM images of enamel surface morphology of the four groups are shown in Fig. 2. Group-2 (SDF) and Group-3 (Laser + SDF) exhibited a relatively smooth enamel surface, whereas Group-1 (Laser) showed minor mineral loss in the enamel rod sheath area. Enamel rods were severely exposed in Group-4 (No treatment) with interrod enamel largely dissolved.

### 3.4. Antibacterial test

SEM images (Fig. 3) showed the biofilm architectures on the enamel surfaces with different treatments. The enamel surface in the Group-4 (No treatment) was found fully covered with *S. mutans* biofilms. Enamel treated with laser only or laser plus SDF displayed more free regions without cells adhered. In addition, SDF alone or laser plus SDF could destroy the

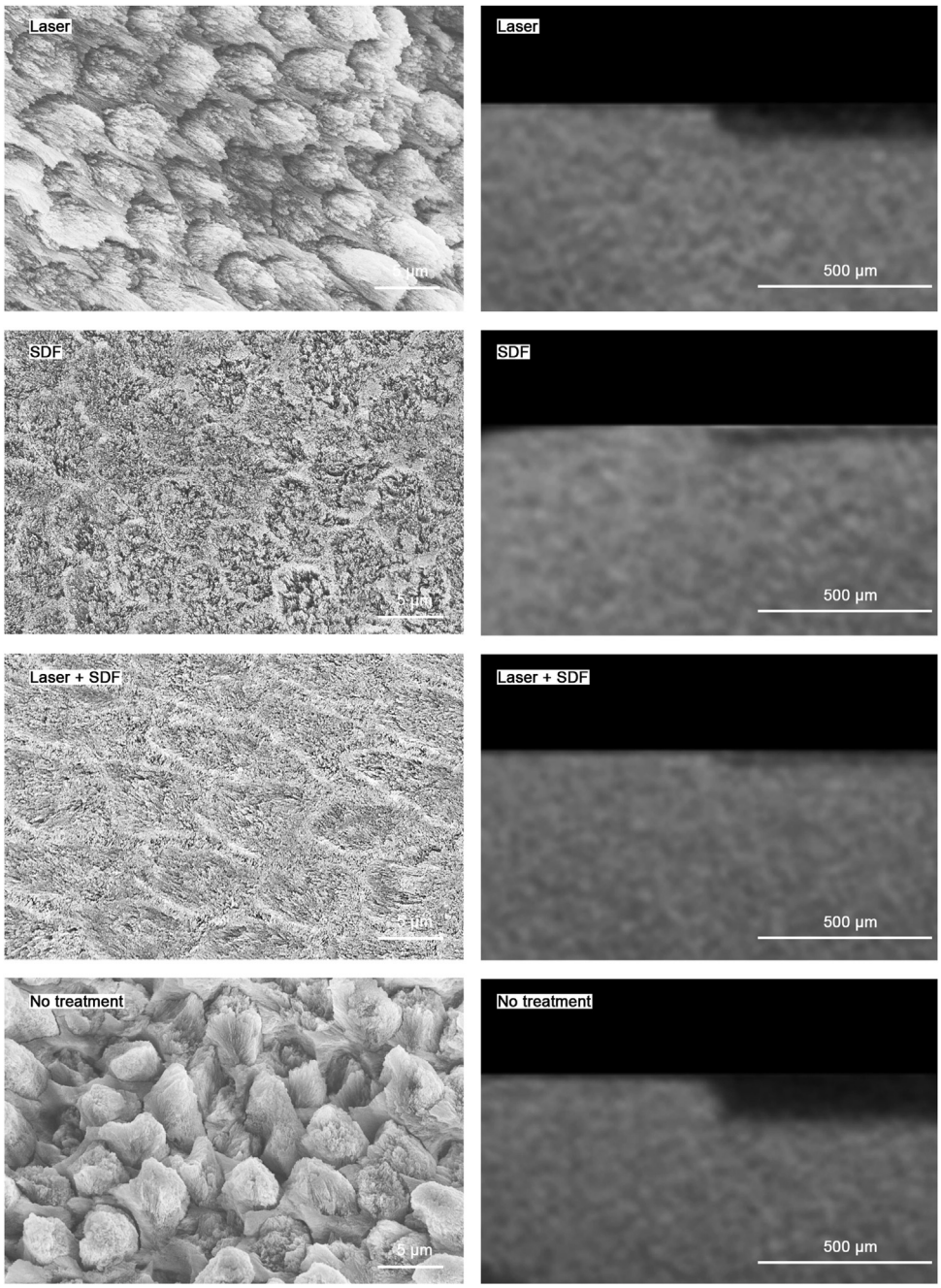


**Fig. 2 – Scanning electron micrographs of enamel surface morphology (left) and microcomputed tomography images of artificial caries lesion (right).**
**SDF - silver diamine fluoride; Laser - 9.3-μm carbon dioxide laser.**

**Table 2 – Lesion depth, micro-hardness, Ca/P molar ratio, and fluoride weight percentage (mean ± SD) of the four treatment groups.**

| Assessment of demineralisation | Group-1 Laser | Group-2 SDF | Group-3 Laser + SDF | Group-4 No treatment | *p* Value Post-hoc test |
|---|---|---|---|---|---|
| Lesion depth (μm) | 88 ± 21 | 26 ± 11 | 13 ± 9 | 115 ± 25 | $p < 0.001$ Group 2,3 < 1 < 4 |
| Micro-hardness value | 255 ± 13 | 253 ± 25 | 294 ± 21 | 173 ± 46 | $p < 0.001$ Group 4 < 1,2 < 3 |
| Ca/P molar ratio | 1.66 ± 0.03 | 1.63 ± 0.03 | 1.67 ± 0.02 | 1.57 ± 0.02 | $p < 0.001$ Group 4 < 1,2,3 |
| Fluoride weight percentage | 0.81 ± 0.26 | 1.62 ± 0.33 | 1.51 ± 0.48 | 0.91 ± 0.11 | $p < 0.001$ Group 1,4 < 2,3 |

SDF - silver diamine fluoride; Laser - 9.3-μm carbon dioxide laser; Ca - calcium; P - phosphorus.

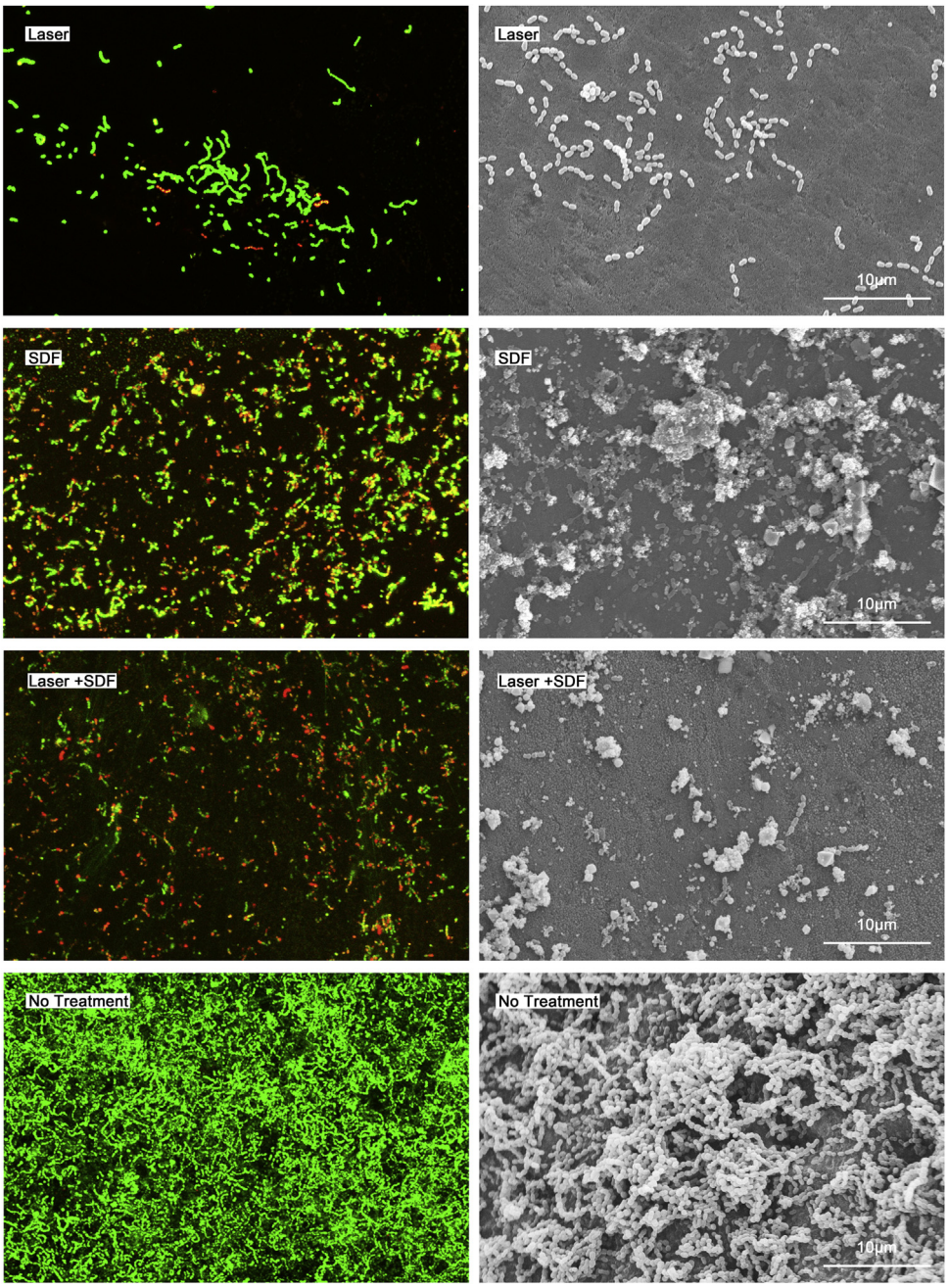


**Fig. 3 – Confocal laser scanning microscope images (left, ×100) and scanning electron micrographs (right) of the four treatment group.**
**SDF - silver diamine fluoride; Laser - 9.3-μm carbon dioxide laser. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)**

*S. mutans* biofilms and morphologically intact bacteria were rarely spotted.

CLSM pictures (Fig. 3) found a large number of living cells with the green fluorescence in Group-4 (No treatment). In comparison, more red fluorescence representing dead cells was found on the enamel surfaces treated with SDF alone or Laser + SDF. Group-4 (No treatment) had a thick biofilm structure in the reconstructed CLSM pictures (Fig. 4) compared to the other three groups. Biofilms in the Laser + SDF treatment group showed obviously reduced thickness, which indicated the biofilm structure was disrupted.

The Log CFUs values of *S. mutans* for the four groups are shown in Fig. 5. The results revealed that the bacterial count decreased in the biofilm structures of all the treatment groups compared to the negative control group. Group-2 (SDF) and Group-3 (Laser + SDF) presented a significantly lower Log CFUs than Group-1 (Laser), whereas no significant differences were found between Groups 2 and 3.

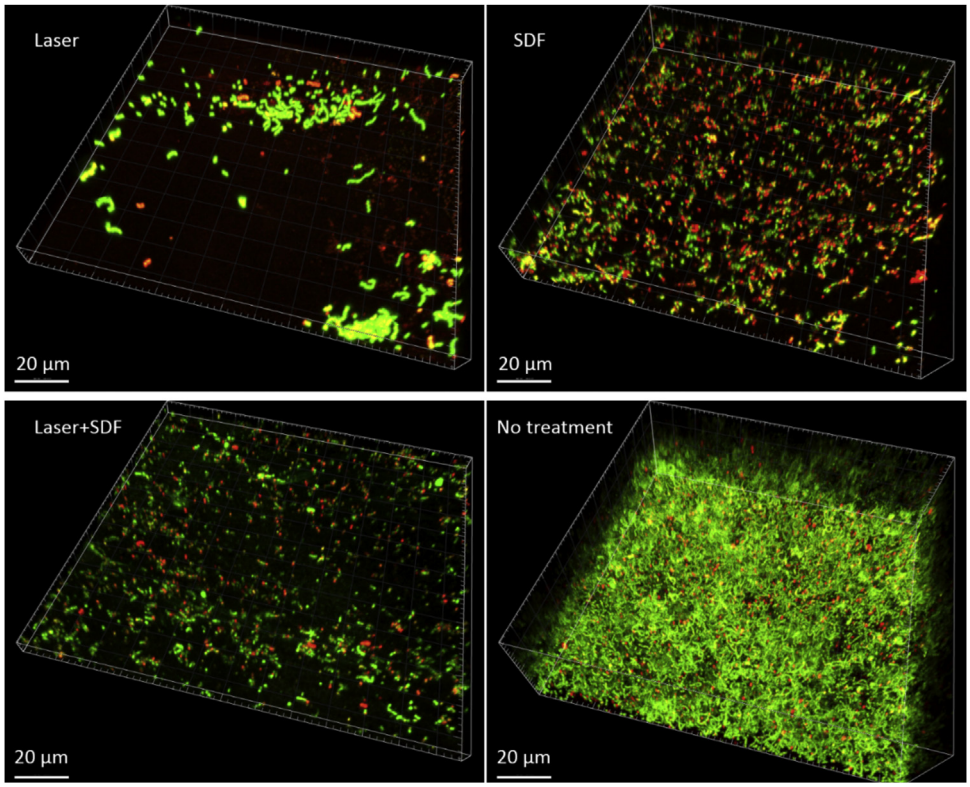


**Fig. 4 – Reconstructed confocal laser scanning microscope images and bacterial counts of the four treatment groups. SDF - silver diamine fluoride; Laser - 9.3-µm carbon dioxide laser.**

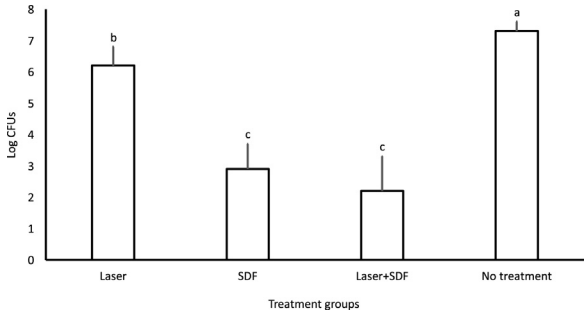


**Fig. 5 – The Log CFUs of *S. mutans* for the four treatment groups.**
**SDF - silver diamine fluoride; Laser - 9.3-µm carbon dioxide laser; CFUs - colony-forming units.**
**Groups sharing the same letters are not significantly different ($p > 0.05$).**

## 4. Discussion

Dental enamel, containing 3% to 6% carbonate content by weight, is a calcium deficient and highly carbonated apatite [12]. The characteristics of enamel make it more soluble in acid than pure hydroxyapatite. The $CO_2$ laser generates wavelengths primarily on 9.3, 9.6, 10.3 and 10.6 µm. These wavelengths lie in the infrared region, which coincides with the main absorption bands of the carbonated and phosphate groups of hydroxyapatite [9]. This allows the laser light to be strongly absorbed by the enamel when the enamel surfaces are irradiated with $CO_2$ lasers. In this study, we used 9.3-µm $CO_2$ laser and SDF to enhance the resistance of enamel against caries attack. The specific wavelength of the laser matches well the absorption characteristic of hydroxyapatite in enamel and its absorption depth of enamel is comparatively shallow. This study used a prototype of 9.3-µm $CO_2$ laser machine which was not available in the market. To the best of our knowledge, this is the first study reporting the effect of the combined use of a 9.3-µm $CO_2$ laser and SDF for preventing enamel demineralisation and inhibiting cariogenic bacteria.

The initial step of biofilm formation is bacteria adhesion on the tooth surfaces. Thus, it is essential to well understand bacteria-surface interactions for biofilm control. The composition and formation of biofilm varies on different surfaces. The material's surface properties affecting bacteria adhesion and biofilm formation include surface roughness, topography, hydrophobicity and surface charge [24]. These factors may be interrelated and affected by laser irradiation. The hydrophobicity of tooth surfaces plays a very important role in oral bacterial adhesion [25]. Generally, bacterial adhesion can be hindered by changing the hydrophobicity of a surface. A previous study reported that laser irradiation was capable of enhancing the hydrophobicity of enamel surfaces compared to the non-lased ones, and thus less biofilm was found on hydrophobic enamel than on the control [24]. In the present study, the decrease in bacterial adhesion on the irradiated enamel could be related to the increase of the hydrophobicity after laser treatment.

SEM images indicated that the morphology of *S. mutans* cells was not visibly altered in the groups using laser irradiation in this study. This finding suggests that laser irradiation did not cause disaggregation of the bacterial cells in the biofilm. Conversely, the morphological and structural integrity of the biofilm were severely disrupted in the groups using SDF in our study. In addition, the CLSM images showed that the amount of dead cells were more on the enamel surfaces treated with SDF alone or laser plus SDF than that in the laser-treated group and the negative control group. These results demonstrate that SDF could induce disorganisation and disaggregation of the biofilm and inhibit its metabolism and growth.

In this study, we used micro-CT to assess the mineral content of the enamel blocks. Micro-CT is a non-destructive indirect measurement and it needs less manipulation of specimen preparation. Accordingly, we used micro-CT instead of the traditional transverse microradiography or polarized light microscope. The results of micro-CT revealed that the adjunctive use of $CO_2$ laser and SDF could prevent enamel demineralisation against acid challenge and has a better preventive effect than the single use of laser.

Microhardness is another indirect indicator to assess mineral content. Although more fluoride was detected on enamel surfaces treated with SDF only or laser plus SDF in this study, the results of microhardness test showed that laser plus SDF had a significantly higher microhardness value compared to other three groups. This may indicate that adjunctively using $CO_2$ laser and SDF has better preventive effects on demineralisation and can improve the enamel surface's intrinsic property. When tooth surface is irradiated with $CO_2$ laser, the superficial layers of enamel are heated by the absorbed energy and the acid soluble carbonate phase is driven out by the heat to form a less soluble hydroxyapatite [12]. When fluoride is applied at this moment, fluorapatite is formed with less solubility than hydroxyapatite [12]. This may explain why crystallographic changes caused by laser irradiation enhance the SDF effect on prevention of enamel demineralisation.

Basically, the higher the Ca/P molar ratio is, the lower solubility the calcium phosphate compound has. The control group had a significantly lower Ca/P molar ratio compared to other three groups in this study. This suggested that laser alone or SDF only or laser plus SDF could reduce the solubility of calcium phosphate compounds in enamel against acid challenge.

Studies have investigated the adjunctive use of the 9.3-μm $CO_2$ laser and other fluoride agents on prevention of enamel caries, including 1.23% acidulated phosphate fluoride (APF) [26,27] containing 12,300 ppm fluoride ion and a toothpaste slurry of sodium fluoride containing 825 ppm fluoride [12,28]. The result showed that the group adjunctively using fluoride and laser had significantly lower mineral loss than using the laser alone. SDF is a solution with a high fluidity [12]. The 38% SDF used in this study contained a higher concentration of fluoride than that in previous studies. In addition, compared with APF or sodium fluoride, SDF has better antibacterial properties [20,29,30].

Because water is one of the main chromophores of the $CO_2$ laser, water cooling played a key role in controlling temperature and preventing surface change of dental hard tissues. In this study, we applied a thin layer of water film over the surfaces on the enamel surfaces during laser irradiation. Compared with other lasers such as erbium-doped yttrium aluminum garnet (Er:YAG) laser or argon lasers, $CO_2$ lasers are highly absorbed by dental hard tissues [9]. Consequently, the surface temperature after irradiation by $CO_2$ laser will be much higher. The thin film of water could not only prevent dehydration of the enamel surface, but also mitigate the temperature increase induced by the lasers. It also reduced the absorption of surface hydroxyapatite because water could compete with hydroxyapatite as an absorption chromophore.

## 5. Conclusion

This study provided evidence that irradiation with a 9.3-μm $CO_2$ laser alone can prevent the demineralisation of enamel, reduce the adhesion of cariogenic bacteria and disrupt the initial biofilm formation. Moreover, adding SDF can significantly increase the preventive effect and antibacterial ability.

## Acknowledgement

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