CO_2 Laser-irradiation through Topically Applied Fluoride Increases Acid Resistance of Demineralised Human Enamel *in vitro*

Patrick R. Schmidlin[a]/Iris Dörig[a]/Adrian Lussi[b]/Malgorzata Roos[a]/Thomas Imfeld[a]

Purpose: To evaluate the effect of CO_2 laser treatment through topically applied amine fluoride solution on demineralised enamel.

Materials and Methods: Sixty extracted human molar crowns were selected and cut longitudinally into half. One half was subjected to a 10-day pH-cycling procedure to create caries-like lesions, whereas the other was left non-demineralised. The following treatments were randomly assigned (one treatment per tooth, on respective non-demineralised and demineralised matched specimens): exposure to a 1% amine fluoride solution for 15 s without irradiation (group I), irradiation for 15 s with a continuous-wave CO_2 laser (group II), or laser-treatment for 15 s through the amine fluoride solution applied immediately beforehand (group III). Fluoride uptake (n = 30) and acid resistance (n = 30) were determined after treatment. Enamel surface alterations after laser irradiation were monitored using scanning electron microscopy.

Results: In groups I and III, an increased fluoride uptake was detected ($p \leq 0.05$). Laser irradiation through topical fluoride resulted in an increased acid resistance of sound and demineralised enamel specimens in deeper layers ($p \leq 0.05$). In addition, less surface alterations were observed in SEM examination of specimens irradiated through the amine fluoride solution compared with counterparts treated with laser only.

Conclusions: CO_2 laser light application through an amine fluoride solution may be instrumental in enhancing acid resistance of sound and demineralised enamel.

Key words: acid resistance, CO_2 laser, demineralisation, enamel, fluoride uptake

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Several studies have been performed to demonstrate the potential of laser treatment of enamel in caries prevention. Among the different types of lasers used in dentistry, carbon dioxide (CO_2) lasers seem the most appropriate for this purpose because of their absorption potential by dental hard tissues (Fried et al, 1997; Fried et al, 2002). Various explanations for the increased acid-resistance of laser-treated enamel have been suggested, such as decreased enamel permeability, alterations in chemical composition, or a combination of both. The exact mechanisms of enamel diffusion pathways, however, are not yet fully understood. A recent review article summarised the respective literature (Rodrigues et al, 2004).

Hoping for synergistic effects, laser treatment has been combined with the application of fluoride (Tagomori and Morioka, 1989; Hsu et al, 1998; Hossain et al, 2002). Fluoride was applied either before or after laser irradiation using different wavelengths and en-

[a] *Clinic for Preventive Dentistry, Periodontology and Cariology, Center for Dental and Oral Medicine and Cranio-Maxillofacial Surgery, Zurich, Switzerland.*

[b] *Department of Preventive, Restorative and Paediatric Dentistry, University of Bern, Switzerland.*

Reprint requests: *Patrick R. Schmidlin, Clinic for Preventive Dentistry, Periodontology and Cariology, Center for Dental and Oral Medicine and Cranio-Maxillofacial Surgery, Plattenstrasse 11, Zurich 8032, Switzerland. Tel: +41 (0)1 634 08 46. Fax: +41 (0)1 634 43 08. Email: patrick.schmidlin@zzmk.unizh.ch*

ergy densities. There is no consensus to date which type or concentration of fluoride should be used or whether fluoride should be applied before or after laser irradiation. Studies reported better results in caries prevention when using the combined application compared to one treatment alone, i.e. laser or fluoride application (Rodrigues et al, 2004). In a previous study using sound enamel specimens it was shown that simultaneous laser irradiation through topically applied amine fluoride solution led to increased fluoride uptake and acid resistance, combined with significantly decreased structural enamel changes (Tepper et al, 2004). According to the authors' knowledge, however, very limited data are available on the effects of laser treatment in combination with fluoride application on carious enamel.

Therefore, the effect of high-energy CO_2 laser application through topical amine fluoride on demineralised enamel was investigated in the present study.

MATERIALS AND METHODS

Tooth sample preparation and laser treatment

Sixty extracted, non-carious human mandibular molars were selected from the department's collection of extracted teeth. Informed consent had been obtained from all patients before tooth extractions. The protocol of the current study did not in any way influence the treatment plan of any patient. Therefore, this study was conducted in accordance with the ethical guidelines for medical research in the Canton of Zurich and the declaration of Helsinki. Teeth were stored in 0.1% thymol solution before use. After removal of the root, teeth were cut in half longitudinally. These tooth sections were comparable with respect to chemical composition of the enamel structure. Tooth sections were embedded in chemically curing acrylic resin, and the enamel surface was cleaned by using a non-fluoridated polishing paste (Pellex, Hawe-Neos Dental, Bioggio, Switzerland) for 30 s with a standardised load of 200 g. Stereomicroscopic examination of the surface of all specimens was performed to ensure that there was no residual resin remaining on the enamel surface areas. Whereas 60 tooth halves remained untreated, the corresponding 60 were demineralised. A computer-controlled 10-day pH-cycling scheme was used to produce artificial caries-like lesions (Francescut and Lussi, 2006). The demineralising solution, at pH 4.6, contained 50 mmol/l acetic acid, 1.5 mmol/l $CaCl_2$, and 0.9 mmol/l KH_2PO_4. The remineralising solution, at pH 7.0, contained 130 mmol/l KCl, 1.5 mmol/l $CaCl_2$, and 0.9 mmol/l KH_2PO_4 (Mukai and ten Cate, 2002). The pH cycling process started with the demineralising phase for 1 hour. After a 2-minute wash in deionised and distilled water the remineralising phase, which lasted 6 hours, was started. Solutions were changed after 5 days.

Each tooth half was divided in two areas using a flowable composite resin (Tetric Flow, Ivoclar Vivadent, Schaan, Liechtenstein), resulting in four areas of each corresponding tooth. One area of each tooth half, i.e. non-demineralised and demineralised, remained untreated and served for control. The other area was allocated to one of three treatment groups as follows (Fig 1):

- Group I: exposure to a 1% amine fluoride solution (1%, Elmex fluid, GABA International AG, Basel, Switzerland) for 15 s without irradiation.
- Group II: irradiation for 15 s with a continuous-wave CO_2 laser.
- Group III: laser-treatment for 15 s through the amine fluoride solution applied immediately beforehand.

Samples in Groups II and III were treated with a commercially available continuous-wave CO_2 laser (Sharplan 15F, Lumenis Inc., Santa Clara, CA, USA). The laser light wavelength was 10.6 µm at an output of 2 W. The test areas of the enamel samples were irradiated for 15 s by one dentist moving the laser probe tip continuously at a standardised distance of 4 mm from the sample surface. For group III, amine fluoride solution was applied immediately before starting the laser treatment. After treatment, all specimens were rinsed with deionised water for 30 min.

Before and after laser treatment, a polyvinyl siloxane impression (President Plus Jet light body, Coltène AG, Altstätten, Switzerland) was made from each sample for SEM analysis of the enamel surface structure using a replica model (see below).

Evaluation of fluoride uptake

Thirty non-demineralised and 30 demineralised enamel halves were prepared and treated as described above. For each of the areas, a circular enamel area with a diameter of 3 mm was created using an acid-resistant adhesive tape. The adhesive tape was fixed to the specimen and its carrier with polyvinyl siloxane of low viscosity (President Plus Jet light body). For the determination of fluoride content in the enamel surface, specimens were etched with 10 µl 2 mol/l HCl for 5 s, and, in a second step, for another 10 s. This pro-

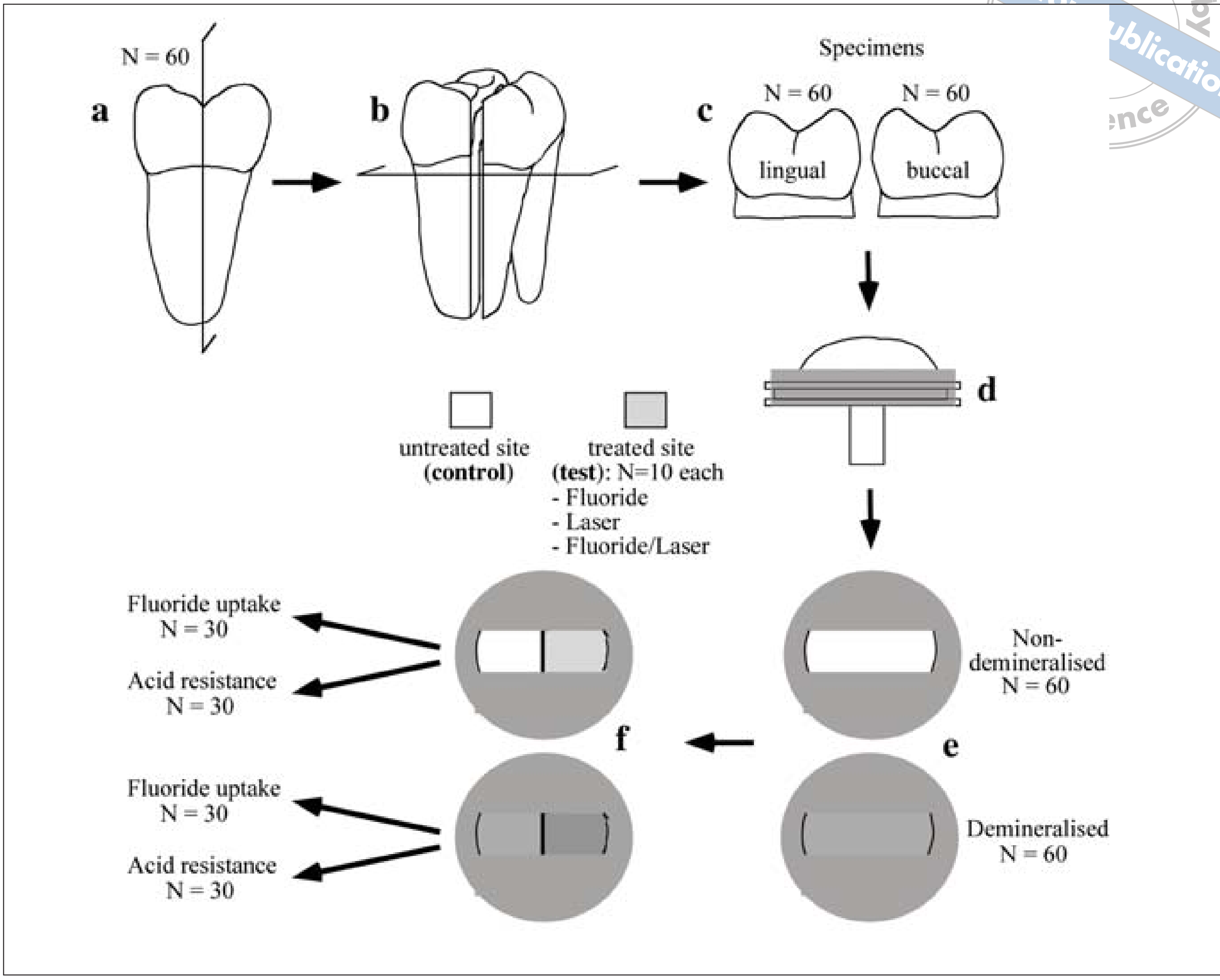

Fig 1 Step-by-step illustration depicting the specimen preparation and group assignment procedure: 60 extracted lower first molars were cut mesio-distally (a) and roots were removed (b and c). Specimens were fixed on SEM mounts and embedded with a self-curing resin (d). One tooth half was subjected to a demineralisation process (e). After initial caries simulation, lingual and buccal crown specimens were divided in two areas, which were then randomly assigned to one of the following treatments: fluoride application alone (group I), laser irradiation alone (group II) or a combination of both (group III). One area was left untreated and served for control (f). Thirty teeth were used for the fluoride uptake and 30 specimens for the acid resistance measurements respectively.

tocol allowed for fluoride determination in the surface layer and in the underlying subsurface stratum. The acid with dissolved enamel was collected using three blotting paper disks of standardised size, which were immediately transferred into 2 ml of TISAB solution (Total Ionic Strength Adjustment Buffer, Orion Research, Boston, USA). For the determination of the fluoride concentration in these solutions, measurements were performed with an ion-selective electrode against blank measurements with paper disks only (Kissa, 1983).

Acid resistance

The remaining 60 tooth halves (30 non-demineralised and their corresponding 30 demineralised counterparts) were used in this evaluation. Tooth quarters were then treated as described above. Each area was covered with adhesive tape with a 3 mm circular area. Samples were fixed on a shaker, and 120 µl of 5 mmol/l lactic acid, pH 3.0, was applied to each specimen. The lactic acid remained on the surface for 5 min under constant shaking. Subsequently, the acid and dissolved enamel components were removed with a

Table 1 Fluoride uptake (ppm fluoride) measured as the difference between paired control and test areas (negative values: increased fluoride concentration in test samples, i.e. fluoride uptake). Statistical differences were analysed (one sample *t*-test, bold p-values represent significant differences p ≤ 0.05).

Group	Non-demineralised			Demineralised		
	Surface layer					
	Mean	95% CI	p-value	Mean	95% CI	p-value
I	-328.5	(-642.9, -14.1)	**0.0423**	-532.9	(-995.7, -70.1)	**0.0285**
II	106.3	(-134.1, 346.7)	0.3432	58.0	(-207.6, 323.6)	0.6330
III	-331.4	(-650.8, -12.0)	**0.0435**	-654.5	(-1203.3,-105.7)	**0.0245**
	1st subsurface layer					
	Mean	95% CI	p-value	Mean	95% CI	p-value
I	-186.9	(-367.4, -6.4)	**0.0438**	-350.2	(-543.2, -157.2)	**0.0027**
II	-69.8	(-224.3, 84.7)	0.3333	-87.1	(-215.8, 41.6)	0.1599
III	-62.8	(-244.5, 118.9)	0.4542	-348.5	(-577.8, -119.2)	**0.0074**

pipette and collected in a test tube. Immediately after acid exposure, the sample surface was rinsed with 50 µl of deionised water, which was also added to the test tube, and the sample surface was dried (Grobler et al, 1990). This procedure was repeated once to investigate enamel dissolution of two consecutive layers (surface and subsurface). Calcium content in these solutions was immediately measured using atomic absorption spectroscopy at 422.7 nm. Phosphorous was masked with strontium chloride (0.25%).

Scanning Electron Microscopy (SEM) examination

Using the polyvinyl siloxane impressions, replicas of the surfaces were cast and bonded to SEM mounts. The mounted replicas were gold-coated and analysed using a SEM (Amray 1810/T, Amray, Bedford, USA) at low magnification (500x).

Statistical analysis

In accordance with the paired design, differences between the values of control and test samples for each group were calculated. For the description of these data, mean values and corresponding 95%-confidence intervals (95%-CI) were given. For the differences between test and control values, one sample *t*-test was used, given the hypothesis that the true difference is equal to zero, i.e. no treatment influence occurred. One-way ANOVA together with post-hoc test by Bonferroni was applied to find the differences between the experimental treatments. Significance was set at 95%.

RESULTS

Fluoride uptake

The results of the fluoride determination are summarised in Table 1. Since values derived from the subtraction of test from control values, negative values indicated a fluoride-uptake, whereas positive values indicated a decreased fluoride content after treatment compared with the untreated control. On demineralised and non-demineralised enamel specimens a significant fluoride uptake could be observed in the subsurface enamel in groups I and III ($p \leq 0.05$). No significant changes in the fluoride content were found after laser treatment alone.

An overview of the comparison between the different treatment regimens is given in Table 2. In the surface layer of the non-demineralised specimens, significant differences could be found between group II and the other treatments ($p \leq 0.05$). No such differences could be found in the second layer ($p = 0.4449$).

On demineralised specimens, group II differed significantly from group III in the first layer ($p = 0.0157$), whereas the second layer showed no significant differences ($p = 0.0518$).

Acid resistance

The results of the acid resistance determination are summarised in Table 3. This was measured by assaying the amount of dissolved calcium from enamel specimens upon exposure to lactic acid. Again the values of the test areas were subtracted from those of the untreated controls. Positive values indicated an increased acid resistance.

Table 2 **Statistical differences among the three treatment groups with regard to fluoride uptake (one-way ANOVA together with post-hoc test by Bonferroni)**

Group	Non-demineralised		Demineralised	
	Surface layer			
	Group I	Group II	Group I	Group II
Group I	-	**0.0252**	-	**0.0157**
Group III	n.s.	**0.0244**	n.s.	n.s.
	1st subsurface layer			
	Group I	Group II	Group I	Group II
Group I	-	n.s.	-	n.s.
Group III	n.s.	n.s.	n.s.	n.s.

n.s., not significant

Table 3 **Acid resistance (calcium dissolution in µg calcium) measured as the difference between paired control and test areas (positive values: increase in acid resistance). Statistical differences were analysed (one sample *t*-test; bold values represent significant differences, p ≤ 0.05).**

Group	Non-demineralised			Demineralised		
	Surface layer					
	Mean	95% CI	p-value	Mean	95% CI	p-value
I	0.11	(-0.09, 0.31)	0.2481	0.21	(0.10, 0.32)	**0.0015**
II	-0.29	(-0.79, 0.20)	0.2076	-0.63	(-1.09, -0.17)	**0.0127**
III	0.35	(-0.12, 0.82)	0.1227	0.30	(-0.08, 0.69)	0.1017
	1st subsurface layer					
	Mean	95% CI	p-value	Mean	95% CI	p-value
I	0.18	(0.05, 0.32)	**0.0118**	0.09	(-0.09, 0.27)	0.2711
II	-0.12	(-0.62, 0.38)	0.6024	-0.15	(-0.36, 0.05)	0.1226
III	0.56	(0.25, 0.87)	**0.0025**	0.64	(0.18, 1.10)	**0.0108**
	2nd subsurface layer					
	Mean	95% CI	p-value	Mean	95% CI	p-value
I	0.08	(-0.09, 0.25)	0.2996	0.29	(-0.01, 0.59)	0.0554
II	0.08	(-0.22, 0.37)	0.5641	-0.02	(-0.31, 0.27)	0.8886
III	0.60	(0.25, 0.94)	**0.0034**	0.75	(0.36, 1.15)	**0.0018**

Table 4 **Statistical differences among the three treatment groups with regard to acid resistance (one-way ANOVA together with post-hoc test by Bonferroni).**

Group	Non-demineralised		Demineralised	
	Surface layer			
	Group I	Group II	Group I	Group II
Group I	-	n.s.	-	0.0006
Group III	n.s.	n.s.	n.s.	0.0002
	1st subsurface layer			
	Group I	Group II	Group I	Group II
Group I	-	n.s.	-	n.s.
Group III	n.s.	0.0039	0.0073	0.0003
	2nd subsurface layer			
	Group I	Group II	Group I	Group II
Group I	-	n.s.	-	n.s.
Group III	0.0058	0.0056	n.s.	0.0008

n.s., not significant

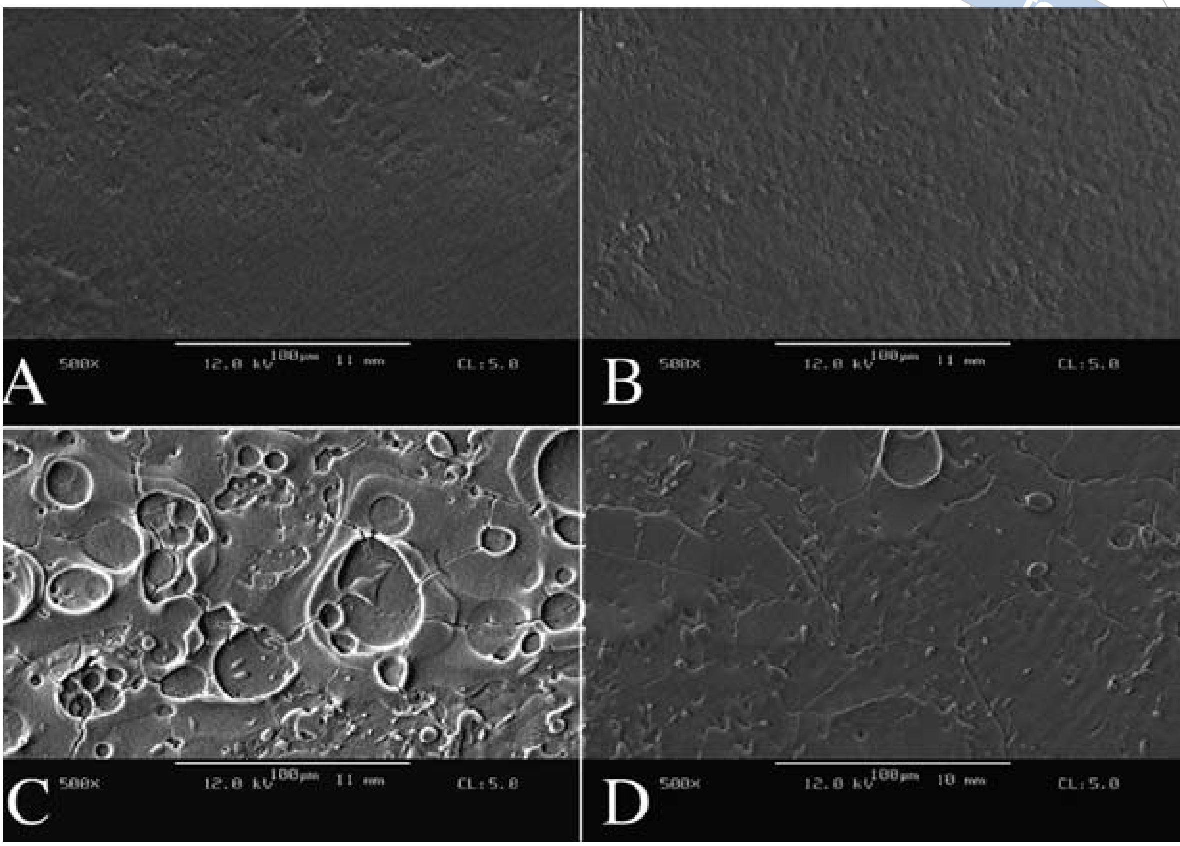

Fig 2 Representative SEM micrographs (original magnification 500x), showing demineralised enamel specimen surface alterations after different treatment modalities. A) Untreated smooth surface (control). B) Fluoride treatment for 15 s (group I) resulting in an etching pattern. C) Laser application alone (group II). Accentuated pattern of mosaic cracks and extensive melting areas were observed. D) Laser-treatment through amine fluoride solution for 15 s. Significantly less surface alterations were visible compared with laser treatment of dry surfaces.

Treatment of non-demineralised enamel had little impact on acid resistance in the surface layer ($p > 0.05$). In the first subsurface layer, however, an increased acid resistance was observed for groups I and III. Whereas fluoride application alone showed no effect in the second subsurface layer, combined laser and fluoride treatment resulted in a significantly decreased acid dissolution ($p \leq 0.05$).

On demineralised samples, statistical changes of the calcium dissolution were only apparent in groups I and II. Whereas fluoride application alone led to a higher acid resistance, laser treatment alone showed the opposite finding, i.e. test samples showed a higher calcium dissolution rate as compared to the untreated control counterparts ($p \leq 0.05$). The combined laser treatment showed no statistical effect in the first layer. However, with regard to both subsequent subsurface layers, a statistically increased acid resistance was found for the combined laser treatment ($p \leq 0.05$).

An overview of the comparison between the different treatment regimens is given in Table 4.

SEM examination

Micromorphological evaluation at magnifications of 500x revealed some irregularities of surfaces of untreated natural enamel. A shallow uniform etching pattern could be observed after fluoride treatment. Laser treatment alone showed extensive moonscape resembling melting areas and clod-like cracks. When amine fluoride solution was applied immediately prior to laser treatment, surface alterations were markedly reduced (Fig 2).

DISCUSSION

Several laboratory studies have investigated the synergistic effects of laser treatment and fluoride application (Rodrigues et al, 2004). In general, combined treatment was considerably more effective than the single treatments. No consensus was found, however, with regard to whether the fluoride application should be performed before or after laser irradiation. A persisting problem of laser irradiation, heating, may hamper surface morphology by melting and crack formation (Ferreira et al, 1989; McCormack et al, 1995). A recent study showed that laser irradiation through topically applied amine fluoride solution increased acid resistance of sound enamel, while simultaneously reducing adverse heating effects (Tepper et al, 2004). Limited data are available on the effects of this combined treatment on demineralised enamel. The present work reproduced and confirmed the positive results obtained with non-demineralised enamel of the earlier study. According to the authors' knowledge, only a few laboratory investigations have studied the effects on caries-like lesions in terms of fluoride uptake and acid resistance. An increased fluoride uptake was also found in demineralised specimens in another laboratory study, which was in line with the results of the present evaluation (Phan et al, 1999). However, this study used a considerably prolonged fluoride application of 5 minutes before laser application. No data were presented concerning the morphological effects of the treatment. In a previous study by the same working group, Featherstone and co-workers showed a complete inhibition of lesion progression in a pH-cycling model (Featherstone et al, 1991). In the latter study, fluoride was applied after laser irradiation. It must be acknowledged that both latter studies used a wavelength of 9.6 µm, which has a better absorption coefficient in hydroxyapatite than the 10.6 µm used in the present investigation. From the literature it is evident that fluoride present during enamel remineralisation increases the remineralisation efficacy and that the remineralised enamel becomes more resistant to further acid attack (Koulourides and Cameron, 1980; Ostrom et al, 1984; Koulourides and Chien, 1992).

Various different laser types, application times, wavelengths, power, demineralisation models, and target distances were used and described in the literature, making it impossible to recommend a standard procedure (Rodrigues et al, 2004). Thus, investigations aiming to establish and validate a standard protocol of application should be performed before this procedure can be recommended for caries prevention in dental practice.

CONCLUSIONS

The present *in vitro* study showed that the combination of laser irradiation with topical application of amine fluoride resulted in higher fluoride uptake and better acid resistance, along with decreased structural enamel disintegration, on both non-demineralised and demineralised enamel. Laser irradiation of enamel through a topically applied amine fluoride solution may thus be useful and effective in the prophylaxis and management of patients at risk for dental caries. Additional studies are necessary to confirm or disprove these findings.

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