Effect of diode laser on enamel fissure system

Morphological and microhardness analysis

Authors_Ali M. Saafan, Samah S. Mehani & Nermin M. Yussif, Egypt

_Introduction

Although a declining incidence of dental caries has been observed worldwide, it is still the most prevalent disease in childhood and adolescence.[1] Several methods of prevention have sought to reduce caries prevalence,[2] such as fluoride application, sealants, preventive resin restorations and antibacterial therapy, which can reverse the caries process.[3] Nowadays, sealing materials are gaining acceptance in the scientific community, although they still present some disadvantages: contamination of the operation field and contraction during polymerisation. These issues have led researchers to investigate alternative solutions in order to overcome these limits.[4]

Wear and marginal loss are still the most prominent drawbacks of conventional sealing materials, which lead to exposure of the previously sealed areas.[5] Hence, failure to achieve a satisfactory bond using fissure sealants may be due to the lack of tag formation following poor etching of the prismless structural lines of the fissure system.[6]

Owing to all of these drawbacks of pit and fissure sealants, attention has been directed to laser and its positive effect on the enamel surface. A wide range of lasers (argon, CO_2, Nd:YAG and Er:YAG) have been used to increase the resistance of the tooth structure to caries. It has been demonstrated that laser can alter the permeability and the crystalline structure significantly, promoting the enamel's resistance to demineralisation.[7] The phenomenon responsible for this effect is related to the chemical and physical changes in the enamel induced by laser. The irradiated enamel surface is subjected to water loss between 80–120°C, to decomposition of the small quantity of organic substance at 350°C, to initial loss of carbonate hydroxyapatite between 400–600°C, and to enamel melting at more than 800–1,000°C. The high temperatures reached in the superficial layers of the irradiated areas of the tooth cause melting of the enamel, which then recrystallises, forming hydroxyapatite crystals larger than the initial ones.[8] Tagomori et al. found that the irradiated enamel surfaces show higher surface roughness in comparison with the untreated ones.[9] Marquez et al. observed that the lased surface usually



Fig. 1_An ESEM image of group two 2 shows the rods (R) and interrod regions (I) at X3,000x magnification. Interrod regions (I) are deprived deviate from the normal crystalline arrangement.

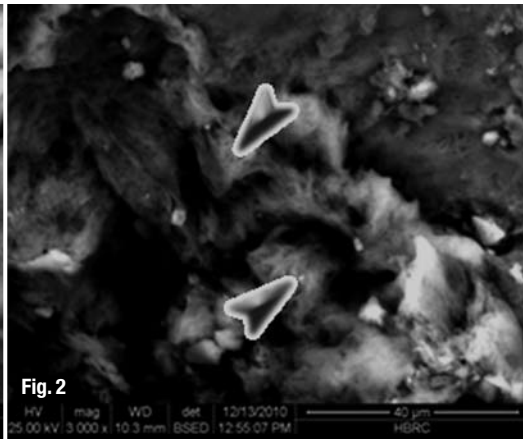


Fig. 2_ An ESEM image of group four 4 shows melting and resolidification of the lateral walls of the fissure and enamel crystals (arrows) at 3,000 x magnification.

Group	1 (control)	2	3	4
Treatment	None	Artificial caries	diode Laser	diode Laser and artificial caries

Table 1_Grouping.

exhibits three layers (from the deepest layer): unaltered enamel crystals,fused crystals and hexagonal hydroxyapatite columns, voids and microcracks on the external surface.[10]

Among the wide range of lasers now used in dentistry, diode lasers offer many advantages that make them quite popular among dentists. Their low cost, small size and ease of use in the oral cavity owing to fibre delivery are important features that favour their use in clinical practice and encourage new studies.[11] Previous studies using diode lasers have demonstrated that the enamel surface of the deciduous teeth underwent melting and resolidification. These changes suggest an increase in the resistance of the enamel to acids, thus possibly playing an important role in the prevention of dental caries.[12]

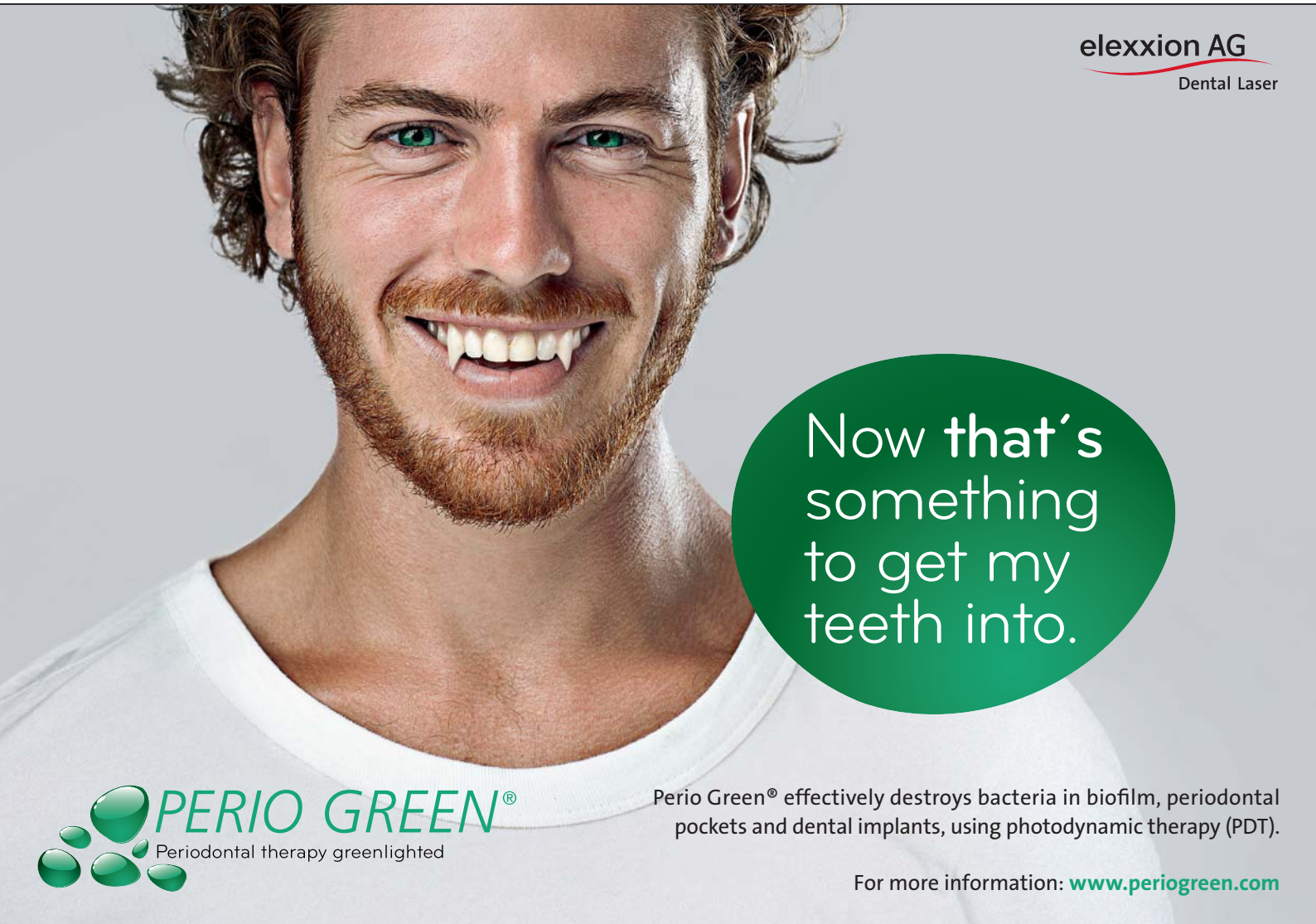
The aim of this article thus is the evaluation of the microhardness and morphological changes that occur in the fissure system of human dental enamel after diode laser treatment in order to examine its sealing and anti-cariogenic effect.

_Material and method

Sample preparation

Forty disease-free recently extracted permanent molars and premolars were used in the experiment. Extraction had been done for orthodontic treatment. Using a diamond low-speed disc, the teeth were sectioned into two halves buccolingually, which were then used in four different groups (Table 1). Each group contained ten teeth. Group 1 (control group) consisted of teeth with normal enamel. The teeth in group 2 were immersed in ar-

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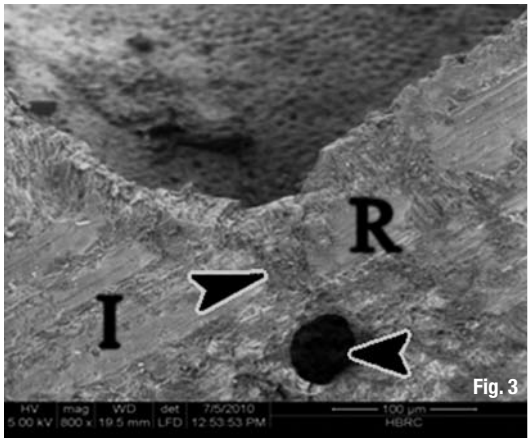


Fig. 3_ An ESEM image of group three 3 shows fusion of the lateral walls of the fissure, leaving retaining the depth of the fissure preserved (black arrow), as well as the rod (R) and interrod regions (I) at 800 x magnification.

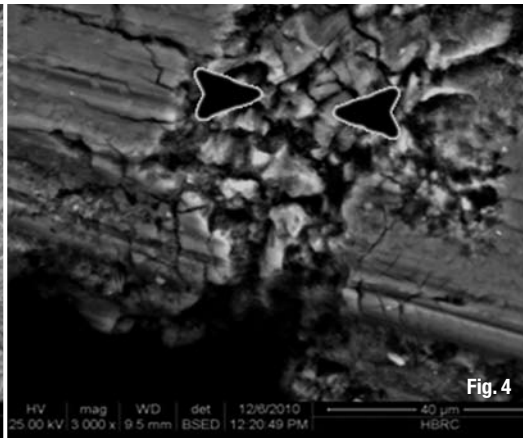


Fig. 4_ An ESEM image of group three 3 shows an accumulation of large crystals with differentof various shapes and sizes (arrows) at 3,000 x magnification.

tificial caries media. Group 3 was subjected to diode laser irradiation. Lastly, group 4 was exposed to diode laser irradiation and artificial caries media. The specimens were kept in distilled water prior to and after examination.[13]

Enamel-surface treatment

In groups 3 and 4, enamel occlusal depressions were irradiated using diode laser irradiation of 980 nm, 2 W for 15 seconds, in contact mode (Quanta System) and an optic fibre transmission system. The fibre tip was positioned perpendicular to the pit and fissure areas. The irradiation was performed by hand, scanning the enamel surface with a uniform motion.[14]

Measurement of thermal changes

Surface and intra-pulpal temperature changes were measured using a thermocouple tester (Fluke 52) in order to evaluate the changes during irradiation.

Artificial caries

The specimens of groups 2 and 4 were individually immersed in an artificial caries media (a media of 50 mmol lactic acid in 6% hydroxyethyl cellulose) with a pH of 4.5 for seven days.[15] The specimens were then washed and kept in distilled water.

Environmental Scanning Electron Microscope analysis

The specimens were examined occlusally and proximally using an ESEM (Inspect S ESEM, FEI). In group 3, ESEM was very useful in examining the specimens before and after in order to confirm the results.

Microhardness measurement

Surface hardness was measured using Vickers microhardness tester (HMV-2 Shimadzu). Measurement was done proximally at the depth of the fissures and at their lateral sides to determine the effect. Indentations were made with the long axis of the Knoop diamond perpendicular to the inner enamel surface laterally and at the depth of the fissures. Each group underwent a load of 19.61 N, applied for 20 seconds, in order to evaluate the variations in surface hardness eventually caused by laser treatment in comparison with unlased enamel. The hardness values were computed automatically.

Statistical analysis

The data was collected and analysed using the ANOVA test. The statistical results were processed by SPSS software (version 17.0, SPSS).

_Results

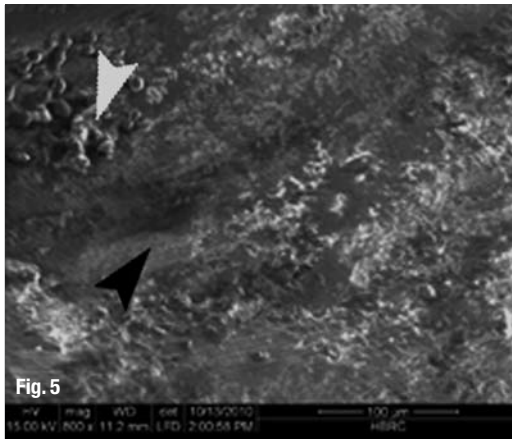


Fig. 5_ An ESEM image of group three 3 shows an occlusal view of the sealed enamel fissure (black arrow) and with molten globules were detected near the irradiated areas (white arrow) at 800 x magnification.

Environmental Scanning Electron Microscope analysis

Total destruction and loss of surface topography, such as the disappearance of the normal elevations and depressions, were clinically observed in the specimens of group 2. Structurally, the surface showed a feather-like or scaly appearance. Few enamel crystalline aggregations reprecipitated on the decayed surface, indicating the demineralisation of enamel. Rod and inter-rod regions due to loss of the surface rodless enamel were detected at the wall of the fissures. The inter-rod regions appeared as voids that deviated from the normal crystalline arrangement as seen in Figure 1. Contrary to

those of group 2, the specimens of group 4 showed a preserved surface structure and morphology of the lased areas. The grooves appeared pitted and intact, while the nearby enamel showed a typical keyhole appearance (rod ends) owing to the loss of the rodless enamel. The lateral walls of the pits exhibited an irregular surface owing to the presence of areas of melted enamel intermingled with carious enamel (Fig. 2). The boundaries between lased and unlased areas were distinct, as the intact lased area could easily be distinguished from the surrounding damaged unlased area.

Morphologically, laser irradiation induced localised enamel fusion of the lateral walls of the fissures in group 3, resulting in a sealing-like effect (Fig. 3). Surface pitting was detected occlusally, indicating the disappearance of the continuous fissures. The lateral walls of these pits revealed a melted homogeneous enamel surface that was masked by multiple enamel granules. Elimination of the defects was accomplished by the accumulation of crystals, which varied in shape and size, forming amorphous and heterogeneous tissue and interrupting the prismatic regions (Fig. 4). Figure 5 shows molten droplets found near the irradiated areas. Occasionally, minimal surface destruction was detected.

Measurement of thermal changes

The measurements recorded only a 1°C elevation in the intra-pulpal temperature and a 67°C elevation in the surface temperature during lasing. A rapid decay of the gained degrees occurred once lasing had been stopped and the temperature returned to normal in less than one minute.

Statistical results

Statistical analysis of the data was done using the ANOVA test. The results were presented as mean ± standard deviation, and a p-value of less than 0.05 was considered statistically significant. The analysis determined that both laser and artificial caries treatments had had statistically significant effects on the enamel microhardness. The degree of demineralisation and the ability of the specimens in each group to resist caries were translated into changes in the microhardness measurements. Table 2 shows the mean Vickers hardness values for the four groups. When comparing all groups to the control group, a highly significant difference was detected ($p = 0.0001$). A post hoc test was done to evaluate the differences among groups regarding the measured normal enamel scores. Group 3 (laser) showed a highly significant difference ($p = 0.0001$). In most of the cases, surface hardness significantly decreased after artificial caries immersion. The contrary was found for group 4 ($p > 0.05$), which means that the positive effect was due to laser only (Table 2).

Group	Mean ± standard deviation S.D.
1	566.3 ± 197.265*
2	79.250 ± 33.9894*
3	1577.40 ± 272.517*
4	191.890 ± 22.7996

Table 2_Vickers microhardness tests results.

**Indicatesing a statistical significant valuece.*

_Discussion and conclusion

Caries is a dynamic process consisting of numerous episodes of the loss and gain of minerals (demineralisation and remineralisation) that occur on the enamel surface.[16] Wavelengths in the red and near infra-red regions are poorly absorbed by dental minerals, but are optimally transmitted and scattered through sound enamel.[17, 18] However, in vivo and in vitro studies have described the beneficial effects of lasers in the former spectrum, such as the Nd:YAG laser (1,064 nm) in caries prevention, but they have not been able to describe the cause or the mechanism.[19] Owing to their low absorption coefficient in hard tissue, neodymium lasers are commonly used with a photosensitiser, which increases the absorption of the laser beam at the enamel surface.[20] Diode lasers were used according to the same protocol, but with different parameters (810 nm, 100 mW/cm^2, 30 mW, 90 seconds, continuous wave).[21]

This study was carried out to investigate the sealing ability of the diode laser (980 nm) by measuring changes in the surface microhardness and detecting the morphological changes using ESEM analysis. ESEM has been established as a useful means of non-destructive microscopic examination of the surface areas of naturally moist oral hard tissue, without the need for a complex preparation and drying process. Another advantage is the avoidance of preparation artefacts. The ESEM results revealed a significant difference between lased and unlased tissue.

Among the four groups, the sealing-like effect of the diode laser in the pit and fissure system and an increased surface hardness were found to be the highest in group 3. Laser treatment produced obvious changes in the orientation and shape of the enamel prisms. The resulting homogenous and heterogeneous apatite crystals, different in shape and larger in size when compared with the untreated enamel, might have been due to enamel melting

and resolidification, as well as a loss of prismatic structure, which corroborates the results of Mercer et al.[22] The granules observed in group 3, according to Zuerlein et al.[23] and Fried et al.,[24] can be explained by the release of the inter-rod and the intercrystalline substance (mainly water and carbonate) near the areas directly exposed to the laser action.

Contrary to the results of Bedini, who used Nd:YAG laser, minimal microcracks and surface roughness were detected with comparable parameters but with a different application mode for the laser, from pulsed to continuous wave.[25] According to Bedini, the use of the Nd:YAG laser with low parameters for caries prevention and high parameters for conservative dentistry is recommended.[25] Simulating the clinical condition and using the free-hand technique (continuous wave mode) assisted the reduction of heat accumulation.[26]

Romanos found that the optical penetration depth of a diode laser at a wavelength of 980 nm was smaller than the penetration depth at 1,064 nm and greater than that of the CO_2 laser.[27] For a better understanding of the penetration depth, an absorption spectrum was taken of water. The absorption in water was markedly higher with a diode laser at 980 nm (0.68 cm^{-1}) than at 810 nm (0.12 cm^{-1}), or even using an Nd:YAG laser at 1,064 nm (0.26 cm^{-1}). The smaller penetration depth results in an increased energy deposition in the upper tissue layers.[28]

The low absorption coefficient of the diode laser wavelength in enamel was of great benefit, as it caused rapid elevation of the surface energy during exposure and rapid decay in temperature once lasing had been stopped. There was no adverse effect on the dental pulp. According to Sulieman et al., the increase in the pulp chamber temperature with a diode laser used at 1 to 2 W is below the critical temperature increase of 5.5°C, which is regarded as the threshold value. In order to prevent irreversible pulp damage, this value should not be exceeded.[29]

In the present study, the maximum temperature elevation on the enamel surface was 67°C, with an intra-pulpal elevation of only 1°C. Previous reports have demonstrated the bactericidal effects of 980 nm wavelength diode lasers. The surface temperature detected thus provides sterilisation of the fissures, destroying *Streptococcus bacteria*, the causative agent of dental caries, which die at 60°C.[30] Regardless of the parameters that were used, Souza et al.[12] found smooth surface melting and resolidification at 1 W power for six seconds. These parameters are roughly half the size of the parameters used in this study. So far, no data has been published that describes the effect of the 980 nm high-power diode laser on enamel microhardness.[31]

Massive destruction of the enamel surface and the lowest surface hardness were found in group 2. Contrary to group 2, the lased samples in group 4 appeared to retain the normal enamel architecture in spite of the pH of the artificial caries media being below 4.5. The appearance of the keyholes in group 4 indicated a loss of prismatic structure, corroborating the results of Mercer and Anderson.[22] Group 4 also exhibited irregular lateral walls of the pits, possibly owing to the presence of areas of melted enamel intermingled with carious enamel. In agreement with our results, Fox et al.[32] found an increase in the acid resistance of irradiated enamel, proving the potential of laser in preventing caries. We conclude that a diode laser can simulate the sealing effect of conventional methods to a limited extend by inducing enamel fusion with no harmful effects on the dental pulp._

Editorial note: A list of references is available from the publisher.

_contact laser

Ali M. Saafan
Associate professor
Dental Laser Application Department
National Institute Oof Laser Enhanced Sciences
Cairo University, Giza, Egypt.

Tel.: +20 0100 516 2873
alisaafan@yahoo.com

Samah S. Mehani
Associate professor
Oral Biology Department
Faculty of Oral and Dental Medicine
Cairo University, Cairo, Egypt.

Tel.: +20 0100 114 3525
hemasls@hotmail.comhemasls@hotmail.com

Nermin M. Yussif
BDCs
Dental Laser Application Department
National Institute of Laser Enhanced Sciences
Cairo University, Giza, Egypt.

Tel.: +20 011 827 1929
nermin.yussif@yahoo.com

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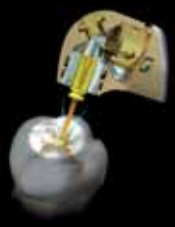
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