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Microhardness and Ultrastructure of Demineralized Gamma-Irradiated Human Enamel after Diode Laser (980 nm) and Fluoride Surface Treatment

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HIGHLIGHTS

- Laser irradiation improved the microhardness of gamma irradiated enamel.
- Fluoride before laser enhanced its effect on gamma irradiated enamel hardness.

Abstract: Quality of life drastically diminished after radiotherapy due to radiation induced oral complications. Fluoride was found to be helpful in decreasing the incidence of radiation caries; however it has not led to elimination of dental caries. Thus, new techniques containing low fluoride concentration or not containing fluoride at all, as laser irradiation, have been studied to prevent the beginning or progression of caries. So the purpose of this study is to investigate the effect of laser surface treatment with or without fluoride on microhardness and ultrastructure of demineralized gamma irradiated enamel; Thirty enamel slabs were allocated into three groups (n=10): G slabs were subjected to gamma irradiation only; GL slabs were subjected to gamma irradiation followed by diode laser and GFL slabs were subjected to gamma irradiation followed by fluoride then diode laser. Slabs

were then exposed to demineralizing solution for 72 hours. Examination of slabs was performed using vickers microhardness test and scanning electron microscope; The lowest microhardness was recorded in group G, while inGL and GFL groups it significantly increased. Scanning electron microscope revealed a pronounced loss of central prism core and retention of prism peripheries in group G. Confluence of prismatic and interprismatic structures in GL slabs and irregular rough surface with prismatic boundaries conservation in GFL slabs were detected. Applying laser improved the microhardness and counteracted the adverse effect of gamma radiation. Adding fluoride before laser irradiation had a marked effect on microhardness..

Keywords: laser; fluoride; gamma irradiation; demineralization; microhardness.

INTRODUCTION

Exposure of the head and neck to ionizing radiation at the therapeutic dose usually caused the occurrence of both acute and chronic oral complications among them xerostomia, mucositis, candidiasis, soft tissue necrosis, progressive periodontal attachment loss, osteoradionecrosis, trismus and radiation caries [1]. Radiation caries with its sudden onset and quick progression could induce complete dentition devastation during the first year post- irradiation [2].

Fluoride is the most widely used method for resisting caries [3]. There are three principal mechanisms through which fluoride works: first it promotes enamel acid resistance, second it reinforces lesions remineralization and third it interferes with microorganisms by prohibiting the metabolism and enzymatic process of such cariogenic organisms, thus preventing them from generating acids [4]. Fluoride varnish has been found to be efficient for being used with high risk patients. However according to the American Dental Association (ADA), it is necessary to apply fluoride varnish 2-4 times per year at three to six months intervals [5]. Fluoride varnish application in head and neck cancer patients was found to be helpful in decreasing the incidence of radiation caries [6].

Recently, great attention is given to laser surface treatment of dental enamel. The physical and chemical changes induced by laser irradiation for enamel is promising with regard preventing its demineralization [7]. These changes depend on many factors including the absorption properties of the tissue [8], laser type and parameters [9]. There are contradictory reports about caries prevention effect of laser. Some studies did not find any significant difference between lased and non lased groups with respect to the enamel demineralization [10,11]. However, other studies, using different kinds of lasers, have demonstrated the potential of laser pre-treatment of enamel in inhibiting subsequent artificial caries-like lesions in the laboratory [12,13]. Diode laser combined with topical fluoride application seems to be a promising preventive method for dental caries and for the immediate fluoridation of teeth [4].

Accordingly the aim of this study was to investigate the effect of diode laser (980 nm) surface treatment alone or combined with fluoride on the microhardness and the ultrastructure of demineralized gamma irradiated human enamel, and to find out whether it was helpful in the improvement of gamma radiation adverse effects on human enamel or not.

MATERIAL AND METHODS

Sample preparation

A total of 30 enamel slabs were obtained from 10 sound human molar teeth that were extracted from patients with age range from 30 to 40 years for periodontal reasons at the clinic of the National Centre for Radiation Research and Technology; Atomic Energy Authority, Cairo, Egypt, with informed donor consent under an ethics-approved protocol (11H/18) of the Research Ethics Committee at National Centre for Radiation Research and

Technology; Egyptian Atomic Energy Authority. The criteria for teeth selection was: free from any visible cracks or fractures, free from previous restorations or decay, free from enamel malformations [14] and no pre-treatment of any chemical agent such as peroxide, as it might alter the enamel surface [15]. Teeth were thoroughly cleaned immediately after extraction and then were stored in distilled water at 4 °C until use to avoid teeth dehydration, and the time elapsed after extraction till use was no longer than three months [16].

Enamel slabs were randomly allocated into three groups according to the type of applied treatment (n=10). Group G where enamel slabs were subjected to gamma irradiation only. Group GL in which enamel slabs were exposed to gamma irradiation followed by diode laser (980 nm) surface treatment. Group GFL was similar to the previous one except for fluoride application before laser surface treatment.

Gamma-irradiation exposure

In this study, all enamel slabs received 60 Gy of gamma radiation (2 Gy/day/5 days/ week/ 6 weeks) at a dose rate of 0.708 rad/sec at the time of the experiment. Gamma irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt, using Cesium 137 irradiation unit (Model: Gamma cell 40, Canada). After irradiation slabs were stored in saline solution at room temperature in order to avoid drying out of the enamel and any falsification of the results [17].

Diode laser application

Twenty-four hours later, enamel slabs of groups GL and GFL were irradiated for 60 seconds by diode laser of 980nm (Model: Quanta c 980 nm, Italy) at continuous mode and power of 1W. The used optic fibre transmission system was 320 nm. The fibre tip was positioned perpendicular to the surface of enamel; in contact mode and irradiation was performed by hand, scanning the enamel surface with a uniform motion [18]. Laser irradiation was performed at the National Institute of Laser enhanced sciences (NILES). Following laser irradiation slabs were stored in saline solution at room temperature for 24 hours before artificial caries induction.

Fluoride application

Fluoride application was carried out by subjecting each enamel slab of GFL to Enamelast fluoride varnish (syringe application) by Ultradent. Enamelast is a flavoured, xylitol-sweetened, 5% sodium fluoride in a resin carrier. Fluoride varnish was applied using a micro-brush; then left on the slab for 1 minute then the excess was gently removed.

Artificial caries induction

A window measuring 3 mm x 3 mm was created on the outer surface of each enamel slab and the remaining portion of each tooth was painted with two layers of an acid resistant nail varnish (Yolo, Egypt). Artificial caries-like lesions were created on the exposed enamel by suspending four slabs into a glass tube containing 20 ml of demineralization solution, for 72 hours, in an incubator at a temperature of 35° C. The composition of the demineralizing

solution was 2.2 mM CaCl₂; 2.2 mM NaH₂ PO₄; 0.05 M Lactic acid and 0.2 ppm Fluoride and was adjusted with 50% NaOH to a pH 4.5 [19].

Microhardness evaluation

After artificial caries induction, the specimens were immediately subjected to microhardness evaluation. Surface microhardness was determined using Digital Display Vickers Micro-hardness Tester (Model HVS-50, Laizhou Huayin Testing Instrument Co., Ltd. China) with a Vickers diamond indenter and a 20X objective lens. A load of 200g was applied to the surface of the specimens for 15 seconds [15]. Three indentations (equally placed over a circle and not closer than 0.5 mm to the adjacent indentations) were made on the surface of each specimen. The diagonal lengths of the indentations were measured by built in scaled microscope. Micro-hardness was obtained using the following equation:

$$HV=1.854P/d^2, \quad (1)$$

where, HV is Vickers hardness in Kgf/mm², P is the load in Kgf and d is the length of the diagonals in mm.

Electron microscope evaluation

After microhardness evaluation, specimens were stored in saline solution at room temperature for another 24 hours. For electron microscopic evaluation, representative samples were air dried, mounted on aluminium stubs with the help of adhesive films and further dried in vacuum before sputter coating with gold. Gold sputter coating was carried out under reduced pressure with the help of a sputtering device (JEOL JFC-1100E ion, Japan). The gold-coated samples were then examined under SEM (JEOL JSM-5400, Japan) operated at 30 kV with magnification 200x power [20].

Statistical analysis

Data were collected, tabulated and analyzed using Statistical Package for the Social Sciences (SPSS) version 23.0 software (SPSS Inc, Chicago, IL, USA). ANOVA test was used to compare the overall differences in micro-hardness results among the three groups. Post hoc Tukey test comparison test was done to determine the main effective group. A *p*-value less than 0.05 (*p* < 0.05) was considered statistically significant.

RESULTS

Comparing the obtained hardness results of enamel slabs of different groups showed that the greatest mean value was recorded in group GFL, then group GL (Table 1). The lowest mean value was recorded in group G (Table 1). One way analysis of variance (ANOVA) test revealed an extremely significant difference between all groups (*p* < 0.0001). Results revealed significant difference between the control group (G) and either GL (*p* < 0.01) or GFL (*p* < 0.01) (Table 1) indicating marked increase in hardness due to laser application alone or in combination with fluoride. Additionally, the mean difference between GL and GFL was significant (*p* < 0.01).

Table 1. The hardness (Kgf/mm²) of enamel slabs of control and treated groups after demineralization

Groups	Mean ± S.D.	P- Value
G	167.08 ± 9.4 ^c	* 0.0001
GL	186.26 ± 13 ^b	
GFL	212.23 ± 3.7 ^a	

Different letters means significant difference, * means extremely significant

In gamma treated group (G), the caries- like lesion showed a pronounced loss of the central prism core and retention of the prism peripheries. This resulted in accentuated prism architecture and markedly porous surface (Figure 1, A). The caries- like lesion in GL slabs subjected to gamma then diode laser irradiation revealed areas showing confluence of prismatic and interprismatic structures alternating with areas showing surface irregularities and marked pitting. The prismatic structure was indistinct. Adjacent areas that were not subjected to acids showed a regular smooth enamel surface (Figure 1, B). The caries- like lesion in GFL slabs subjected to gamma irradiation then fluoride followed by diode laser revealed an irregular rough surface with loss of enamel structure predominantly in the areas of prism centers with conservation of the prismatic boundaries. These areas alternated with other parts showing confluence of prismatic and interprismatic structures and fine porosities (Figure 1, C).

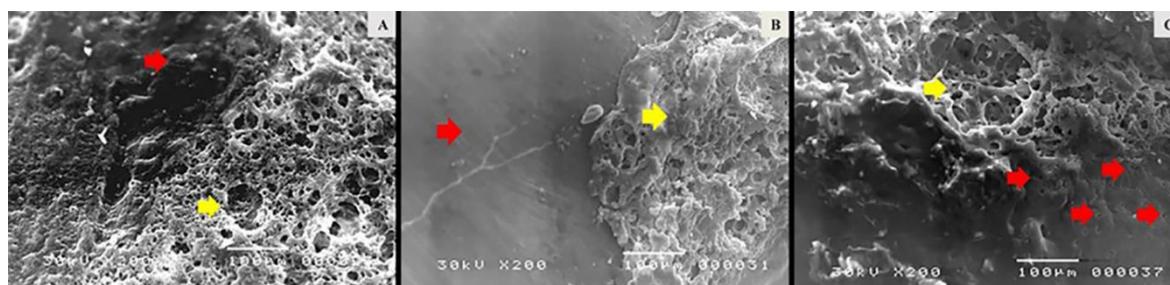


Figure 1. Scanning electron micrograph of enamel slab (A) of G group showing total loss of the prismatic structure (red arrow), variable-sized deep pores separated by thin irregular prismatic boundaries (yellow arrow), (B) of GL group showing indistinct enamel prisms alternate with areas showing confluence of prismatic and interprismatic structures (yellow arrow), smooth regular enamel surface (red arrow) and (C) of GFL group showing termination of enamel prisms on enamel surface with central prism cores appearing as slight depressions and the prism peripheries being elevated (yellow arrow), no exposure of prism cores and very fine porosities (red arrows).

DISCUSSION

In this study, a total dose of 60Gy was fractionated as 2 Gy per day for five days a week for six weeks. The radiation dose normally used to treat head and neck tumors, ranging between 50 and 70 Gy over a period of between five and seven weeks. Doses fractionation allow time for the tumors cells to oxygenate between radiation sessions so make them more radio sensitive ending with enhanced radiotherapy efficacy [21].

Radiotherapy associated complication could be minimized mainly through development of oral care protocols including regular inspection and fluoride treatment before and throughout the therapy period in addition to follow up developed lesion during the therapy[1]. However fluoride role in combat caries is well recognized, global accepted protocol for radiation caries management has not been established yet [22]. Recently, laser treatment shows a promising effect to modify the tooth surface thus reinforce its resistance to demineralization [23]. One of advantageous lasers is diode that has small size, simple application through its fiber optic as well as low cost [24]. Moreover, application of diode laser combined with topical fluoridation has a synergistic effect through enhancing fluoride binding to enamel surface [25]. However; the majority of these researches have been performed on sound enamel surface while gamma-irradiated human enamel has been not yet studied.

Vickers microhardness test was used as it was helpful in detecting the changes in the enamel and dentin, especially following demineralization and remineralization processes [26]. Scanning electron microscope was used to assess the ultrastructure changes of gamma irradiated enamel induced by laser alone or combined with fluoride treatment.

According to the results of this study, there was a marked increase in the hardness of gamma irradiated enamel due to laser application alone where enamel hardness reached 186.26Kgf/mm² and a significant difference ($p < 0.01$) was reported. Fluoride treatment before laser application significantly increased enamel hardness as compared to those of gamma-irradiated slabs ($p < 0.01$) and those of laser treated slabs ($p < 0.01$). Our result was similar to Pavithra *et al.* who found that diode laser (980 nm) irradiation significantly increased the hardness of pit and fissure enamel [27], and Esteves-Oliveira *et al.* who stated that CO₂ laser (10600 nm) increased enamel caries resistance [23]. Moreover, application of fluoride varnish and diode laser irradiation significantly increased enamel microhardness [28], completely inhibited the development of caries lesions in primary teeth [29], and significantly increased the fluoride uptake in comparison to the control group [30]. Increased enamel resistance to demineralization upon diode laser irradiation could be attributed to surface melting and enamel hydroxyapatite crystals re-crystallization [31], decreased enamel permeability and solubility [32], and calcium fluoride deposition [33]. In the presence of fluoride, laser treatment increased the amount of both fluoride loosely bonded to the enamel surface and strongly coupled with enamel crystalline structure [4,30] and induced micro cracks and micro spaces in the enamel surface that facilitate fluoride incorporation [34].

In the present study, SEM analysis demonstrated that gamma-irradiated enamel slabs subjected to demineralization exhibited total loss of the prismatic structure and variable-sized deep pores separated by thin irregular prismatic boundaries. While laser treated slabs showed confluence of prismatic and interprismatic structures and areas of marked pitting. Fluoride application before laser irradiation augment its effect as it limit the enamel loss to prism centers with conservation of the prismatic boundaries.

In accordance with our results, El-Faramawy *et al.* found total loss of the prismatic structure with porous areas of enamel irradiated with 6Gy gamma radiation [35]. Enamel irradiated with Nd:YAG laser (1.2 W power) developed glazed areas, cracks and craters

probably due to an increase of temperature up to the sublimation point of the material that, during the subsequent solidification, completely changed its initial structure [36]. Similarly, CO₂ laser irradiation of enamel slabs at power intensities of 6 J/cm², 10 J/cm², and 11.5 J/cm² induced cracking, melting, and fusion [37]. The positive effect of laser regarding demineralization resistance could be attributed to the melting and fusion of hydroxyapatite crystals [31].

CONCLUSIONS

Enamel slabs subjected to gamma irradiation only showed the lowest values of surface microhardness. Applying laser improved the microhardness and counteracted the adverse effect of gamma radiation. Adding fluoride before laser irradiation had a marked effect on microhardness.

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