

enhance its resistance to demineralization, as laser change its morphology by melting and recrystallizing process. The main objective is to test the effectiveness of laser treatment using pulsed Nd-YAG 1064 nm wavelength on acid resistance of human enamel in vitro. Materials and Methods: Ten human caries free teeth, extracted for orthodontic and periodontal reasons were used in this study. Q-switched Nd:YAG laser Eighteen sectioned teeth samples were divided into six groups, each was treated by different laser energy fluency. Micro-hardness, Surface roughness, Ca/P ratio after determination, Demineralization and Remineralization tests were performed. The results showed, significant increase in microhardness and roughness with increasing laser fluency. Increase in calcium / phosphorus ratio was seen compared to the control. The weight test results showed a significant acid resistance improvement of the laser-treated enamel surface.

KEYWORDS : Tooth enamel, Nd-YAG, Acid resistance

INTRODUCTION

Dental caries is a worldwide problem, considered as the prevalent disease during childhood and adults (Florin et al.,1990). Organic acids are penetrated by acidogenic bacteria present in dental plaque and demineralization process starts when pH decreases lower than 5.5 (Loeshe and Microbiol, 1986). Over the last few decades, a considerable decline in dental caries is seen due to the extensive use of fluoride in drinking water, salt, and tablets, however topical application of florid found to be more efficient during childhood than oral administration (Nammour et al., 2003). The effects of fluoride on dental hard tissues are inhibition of bacterial metabolism, inhibition of demineralization when PH is lowered and enhances remineralization through which a low-soluble fluorapatite-like veneer forms on the remineralized crystals (Featherstone, 1999). Although fluoride is a powerful treatment for the prevention of tooth decay, however; some individuals are still develop pit and fissure caries after application of fluoride (Winston and Bhaskar, 1998), therefore; a new methods to control this disease completely is still necessary (Florin et al., 1990). Lasers has been used as an alternative to fluoride, tested for improving dental enamel properties in order to enhance its resistance to demineralization (Zezell et al., 2009). The preventive effect of laser irradiation on dental enamel against dental caries has been reported since the 1960s and ruby is the first suggested laser used for inhibition of dental caries (Stern and Sognaes, 1972), since then several studies has been reported in this respect. It has been found that enamel treated with laser shows more resistant to demineralization and caries (Nelson et al., 1987; Hossain et al., 2001), as laser change its morphology by melting and re-crystallizing process. Anderson et al recorded 15% reduction in demineralization after laser irradiation of teeth (Anderson et al., 2000). Several types of lasers have been used to reduce teeth demineralization with different parameters, carbon dioxide laser (Stern et al., 1972), Er:YAG, 2.94µm (Liu et al.,2012), Nd:YAG laser,1.064µm²⁰ (Majori et al.,2005), CO2 and Nd-YAG (Tsai et al., 2002). The mechanism by which laser act on the tooth surface is that during heat generation by laser irridation, melting of hydroxyapatite occur and it looses carbonate, up to a complete loss, so it become more resistant to demineralization (Marquez et al., 1993; Huang et al., 2001). In this study we will use pulsed Nd-YAG (1064 nm) laser with different energy fluences to modify enamel surface, our main objective is to test the effectiveness of laser treatment using pulsed Nd-YAG on in vitro acid resistance of human enamel,

with the specific objectives to test enamel microhardness, to study enamel topography, enamel surface roughness, grain size and Ca/P ratio. Our hypothesis is sated that there is significant effect of Nd-YAG laser irradiation on dental enamel modification toward increasing its resistance to demineralization, hens preventing dental caries.

MATERIALS AND METHODS

Study design

A randomized controlled study was conducted in Technology University laboratories in a period between March 2018-March 2019.

Samples

Ten human caries free teeth, extracted for orthodontic and periodontal reasons were used in this study. The teeth were seated in a plastic mold with their buccal surfaces exposed, an epoxy resin and hardener mixture were poured on them. After the mixture is solidified, the mold removed and the teeth sectioned using a low speed diamond wheel saw under water cooling. The samples were polished; using (1200-3000) Amery carbide papers and grinder polisher rotating at (150-300) r/min speeds. A cloth and 0.05 polishing alumina were used together with low speed metallurgical polisher as a final polishing stage. The samples were then put in a distilled water, cleaned by an ultrasonic cleaner for 5 minutes, and stored in distilled water in the laboratory.

Lasing procedure

Q-switched Nd:YAG laser (HUAFEL –China); supplying 1 Joule, 9 ns, 0.8 mm diameter single pulses at 1064 nm wavelength was used to irradiate the surfaces of teeth samples in a non-contact mode. 10 cm focal length was utilized to change the laser energy fluence values by working under focused and unfocused laser beam. Eighteen sectioned teeth samples were divided into six groups A, B, C, D, E, and F, each was treated by different laser energy fluence (table 1).

Table 1:	Samples	treated	by	different	values	of	energy
fluence							

Groups	Energy (mJ)	No. of pulses	Laser fluence (J/cm)
A	0	0	0
В	300	1	38.2
C	400	1	50.95
D	500	1	63.69
Е	600	1	76.43
F	700	1	89.17

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Evaluations

Micro-hardness tests:

Digital Micro Vickers hardness tester TH 715, 2008, China equipped with a high resolution optical microscope was employed to evaluate the surface modifications introduced by laser; under 500 g load and 15 sec indentations conditions.

Surface roughness

Surface roughness was analyzed using Atomic Force Microscope (SPM-AA3000, AFM- Contact mode, Angstrom Advanced INC.USA. Tescan, vega3,Czech) to evaluate the surface topographic changes of the laser-treated enamel surfaces.

Ca/P ratio determination

The weight percentages of the laser treated enamel elements were examined using energy dispersive X-ray spectrometer (EDS), (Angstrom Advanced, AIS2300 Multi-function SEM System-USA).The Ca/P ratio of each sample was evaluated, together with calcium and phosphorus distribution.

Demineralization and Remineralization tests

The teeth samples were immersed for 48 hours in an artificially prepared demineralization solution at room temperature. Each sample was weighed before and after this test using 4digit electric balance (Sartorius Lab Instruments GmbH&Co.KG, Goettingen, Germany). The samples were coated with an acid resistance; except for approximately (1.5x1.5) mm window on the treated surface for the demineralization test purposes. The polished samples were washed by deionized water, left on air to dry and be ready for the weight measurement and (EDS) analysis. The samples were kept individually in artificial saliva (used as a remineralization solution) for 48 hours, washed by deionized water, and left to dry in fresh air to be ready for weight measurement and (EDS) analysis.

RESULTS

Microhardness test results

A significant increase in microhardness was seen in lasertreated teeth enamel with different laser energies compared to the control sample and the maximum rises in tooth microhardness was at 600mJ Nd: YAG and 500mJ Nd: YAG respectively, however group F that received 700mJ energy from Nd: YAG laser was excluded from the study because of the cracks (Figure 1).

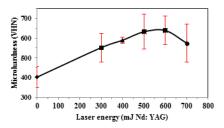


Figure 1: Tooth enamel microhardness versus laser energy

Tooth surface roughness

Increased roughness with increasing laser fluence was seen as laser induced-crystallographic changes, this means larger surface area (Figure 2).

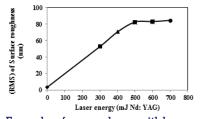


Figure (2): Enamel surface roughness with laser energy

Calcium and phosphorus:

Increase in calcium / phosphorus ratio was seen compared to the control. The demineralized samples of the laser treated groups had higher Ca/P ratios than the demineralized samples of the control group (Figure 3).

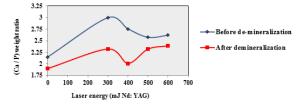


Figure 3: Ca/P weight ratio of all group samples before and after demineralization.

The weight test results showed a significant acid resistance improvement of the laser-treated enamel surface, i.e. reduced solubility, compared to the control samples as shown in Figures (4). The highest improvement ratio for the groups was 362% at 500mJ Nd: YAG treated sample.

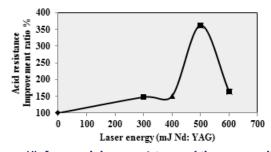


Figure (4): Improved decay resistance of the groups after immersing in demineralization solution for 48 hours.

The re-mineralization test results, demonstrated an increase in the samples weights after immersion in the artificial saliva for 48 hours. This means that the mineral components of the saliva like calcium and phosphate have deposited in the crystal voids of the demineralized enamel. The highest value of remineralization was at 500mJ of Nd: YAG laser (Fig 5).

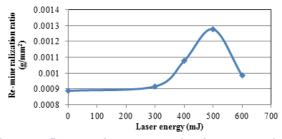


Figure 5: Re-mineralization ratio for the groups after immersing the demineralized samples in the artificial saliva for 48 hours.

DISCUSSION

Modification of hard tissue by laser became popular in dentistry, it has been reported that lasers can significantly alter the crystallinity, permeability, and acid solubility of enamel, which increase its resistance to demineralization. This has an important role in preventive dentistry and enamel etching (Graaff et al., 1993;Hayakawa, 2005;Franke et al., 2006). Caries inhibition by laser varies from 30 to 97.2% according to its wavelength and fluence, the mechanisms of this inhibition remain unclear (Nammour et al., 2003), however; there are conflicting evidences regarding the influence of laser on the structural changes of the dental enamel, this is may be due to increased number of variables used in lasing process such as wave length, power, pulse frequency and duration of irradiation, therefore; the proper selection of parameters for each application is important (Birardi et al., 2004; Lizarelli et al., 2006; Castellan et al., 2007; Banda et al., 2011). To prevent dental caries, chemical changes in the composition or solubility of dental hard tissues must take place, this requires strong absorption and conversion of laser light efficiently to heat without damaging the nearby tissues depending on the temperature rise during irradiation (Ana et al., 2006). The use of laser in dental hard tissues and in carious lesions causes ablation without thermal injury to the surrounding hard tissues such as carbonization or crack formation (Geraldo-Martins et al., 2007). Analysis of Ca/P ratio in the enamel surface and in the demineralizing solution quantitatively is commonly used, which includes mineral loss and determination of calcium and phosphorous dissolution (Tagomori and Morioka, 1989; Bahar and Tagomori, 1994). In this study we set specific parameters for increasing enamel resistance to demineralization with a required depth and without thermal damage to the pulp tissue. Our results showed significant enamel hardening as shown in microhardness test results, this is attributed to the fast laser heating and quenching by the subsequent temperature gradient of a thin layer of the enamel surface during the Nd: YAG laser. The laser-induced shockwave pressure that pushes inside originates from enamel surface ablation, in which phase transformation from solid to vapor state occur leading to plasma formation, which absorbs quickly the laser energy (Michel et al., 2008). The plasma expands and causes a shock wave, which increases the pressure on the enamel surface (Eisner, 1998). The reduction in water solubility of enamel, reduction of carbonate and organic content of the tooth increases tooth microhardness which means lower degree of tooth abrasion and more tooth resistance against carries. This result is in agreement with most of the studies (Florin et al., 1990; Attin et al., 1997; Gary et al., 2003), however others disagree reporting a decrease in microhardness after lasing (Tagomori and Iwase, 1995; Majori et al., 2005). Increased roughness with laser fluence was seen as laser induced-crystallographic changes, the laser intensity treatment can show various morphologic features (Tagomori and Iwase, 1995) and different crystal size (Sato, 1983) on the treated enamel surfaces as thermal recrystallization can increase the enamel crystal size; making the enamel more resistant to demineralization (Lin et al.,2000). This is fully consistent with our results and the published results (Rohanizadeh et al., 1999), which demonstrated a gradual growth in crystallites size. The present findings demonstrate a significant increase in calcium to phosphorus ratio compared to the control, this is due to the re-distribution of mineral components during mineral melting and chemical changes which are responsible for the decrease in permeability and reduction of acid penetration within the internal layers (Tagomori and Iwase, 1995). Usumez et al reported that laser energy modifies the Ca/P mineral ratio and forms less acid soluble compositions leading to lower chances for acid to attack the tooth, this could affect solubility, permeability, or adhesive specifications of the dental enamel (Ari, Erdemir, 2005).

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