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Potential role of Er:YAG laser and fluoride in the dental enamel remineralization: a raman spectroscopy preliminary *ex vivo* study

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ABSTRACT

Background and aims: Dental caries are a widespread oral disease and a serious public health problem, starting by teeth demineralization, which is a loss of minerals such as calcium and phosphate. Modern caries treatment is aimed at preventing the disease progression by teeth remineralization which is a supply of minerals to the enamel. The most popular remineralization method is the treatment of teeth with fluoride. Er:YAG laser has also gained research attention as a method for improving the uptake of fluoride and phosphate by introducing chemical and morphological changes into the structure of enamel but, while some researchers described it as effective, others found no significant effect from its application. This work aimed to further study the effect of Er:YAG laser, alone or combined with fluoride, to dental enamel.
Materials and Methods: Twenty upper central human incisors, extracted for periodontal reasons, were used in the study. Samples were demineralized by acetic acid and divided into four groups: a) control, b) fluoride + Er:YAG laser, c) Er:YAG laser alone and d) fluoride alone. The remineralization rate of teeth was estimated by Raman Spectroscopy.
Results: In comparison with the control group, the phosphate peak's intensity increased notably for the teeth treated by fluoride, but decreased slightly for the teeth treated with Er:YAG laser and with a combination of the laser and fluoride.
Conclusions: With the limits of this study, due to the limited samples number, Er:YAG laser, alone and combined with fluoride, seems to be not effective, at the parameters used, for the enamel remineralization.

Key words: Dental decay, Enamel remineralization, Er:YAG laser, Fluoride, Raman spectroscopy

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**Running Head:*
ER: YAG laser for dental remineralization

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Introduction

Dental caries is the most common disease of dental tissues and the second most common infectious disease after respiratory diseases. Its cause is multifactorial, and its pathogenesis consists of the disruption of the balance between demineralization and remineralization of the teeth [1]. The most critical factors for tooth demineralization are the acid produced by cariogenic bacteria [2] and the loss of enamel's inorganic structure due to erosive processes. One of the most high-risk factors for tooth erosion is excessive consumption of soft drinks with erosive properties able to decrease salivary function and perturb the enamel structure. The inorganic contents of tooth hard structures are lost due to the exposure to acid with a bacterial and nonbacterial origin, so decreasing tooth hardness [4]. The tooth hard tissue loss is caused by two important processes, the dissolution of enamel's hydroxyapatite crystals and the distribution/dissolution of calcium, phosphate, and hydrogen ions into and out of the enamel microstructures. Tooth enamel demineralization is a dynamic process beginning with the formation of incipient subsurface lesions: when the demineralization process persists, incipient clinical lesions result in enamel cavitation [5].

The remineralization of decalcified enamel and the restoration of its surface hardness are two important and challenging aims in dentistry. Topical applications of fluoride have been the primary method for stabilization of early carious lesions through remineralization, and thus, fluoride has had a profound effect on reducing caries prevalence. However, fluoride has only a partial cariostatic and remineralization potential [6] and dental fluorosis remains an issue of concern, especially in young children. Combinations with other methods and non-fluoride agents have been suggested as alternatives in this regard [7]. An adjunct method in the prevention of caries is the use of laser [8]. The caries preventive effect of this laser can be related to reducing the permeability of the enamel by re-crystallization of enamel crystals into larger ones and removal of the enamel inter-rod substance [6]. Laser irradiation causes longitudinal exposure of the enamel rods as a consequence of the micro-explosion, resulting in morphology changes in the enamel through melting and re-crystallizing processes, and creation of larger hydroxyapatite crystals on the surface. This reduces the enamel permeability of acid penetration and modify the mineral composition of its deeper layers [9-11]. Moreover, the heating and melting processes results in significant reduction of carbonate content in hydroxyapatite up to a complete loss resulting in enamel composition that is more resistant to demineralization [12].

Several studies have been performed to observe the behaviour of different laser wavelengths, alone or in combination with fluoride application, on dental enamel remineralization. Thus, Fekrazad *et al.*, [6], Fornaini *et al.*, [14], Zezell *et al.*, [15], Al-Maliky *et al.*, [16] have found a positive effect on the remineralization from the CO_2, Er:YAG, Nd:YAG, and 445 nm diode lasers respectively. On the other hand, Moghadam *et al.*, [17] have found that although the application of Er:YAG laser was effective for remineralization, its combination with fluoride has not shown a significant performance improvement.

Moreover, Delbem *et al.*, [18], Nair *et al.*, [19], and Ulkur *et al.*, [20] have not found any significant contribution from the Er:YAG laser to the demineralization prevention.

The aim of this preliminary ex vivo study is to evaluate, by means of Raman spectroscopy, the effects of Er:YAG laser irradiation, alone and in combination with fluoride, on human permanent teeth.

Materials and methods

Teeth treatment

Twenty human incisors, extracted for periodontal reasons were utilized in this study: they were polished and subjected to the demineralization by immersing them into a demineralizing solution (2.9 g of NaCl, 0.12 g of $CaCl_2$, 0.13 g of NaH_2PO_4, 5cc of NaF (100 ppm), 5 cc of NaN_3 (2% ww), 1.5 cc acetic acid. The pH of the solution was maintained at 4.5. The solution was renewed daily to prevent the accumulation of materials produced by demineralization and the consequent pH change. After 48 hours, the surface of each sample was washed with a syringe containing artificial saliva and placed for storage in the container with the artificial saliva (2.9 g of NaCl, 0.12 g of $CaCl_2$, 0.13 g of NaH_2PO_4, 5cc of NaF (100 ppm), 5 cc of NaN_3 (2% ww)). Subsequently, by means of a diamond burr, a square sample was obtained from the crown of each of them and as shown in Fig. 1., the nineteen samples (one was destroyed and was not able for the research) were divided into four groups: four teeth in group 1 and five teeth in all other groups. After that, each group of teeth has been subjected to the following treatment:

- Group 1 – four teeth were irradiated by the Er:YAG laser (0.5 W power; 2940 nm wavelength; 0.6 mm spot diameter; 120 µs pulse duration, 20 Hz repetition rate)
- Group 2 – five teeth that were not treated and used as a control
- Group 3 – five teeth that were treated by the fluoride at concentrations between 0.5 and 10 ppm and simultaneously subjected to Er:YAG laser (same parameters)
- Group 4 – five teeth treated by fluoride only (same concentration)

After the treatment all teeth have been fixed on a plate and subjected to Raman measurement. Because this is a blind study, the researcher who performed the Raman analysis did not know what was the treatment for each group.

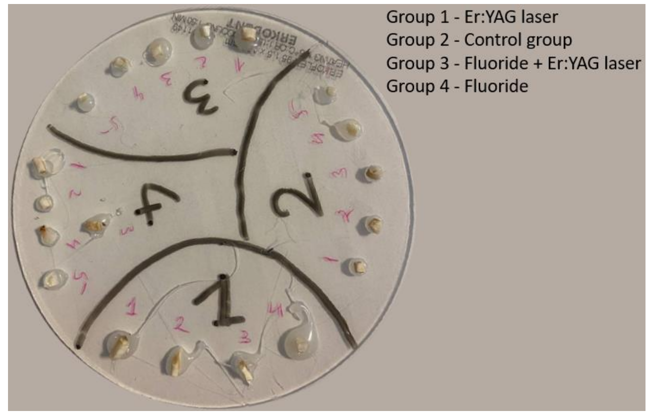

Fig. 1. Four groups of teeth treated by different methods

The laser used in this study is LiteTouch (Light Instruments, Yokneam, Israel), emitting at 2940 nm. It was used in contact mode with the handpiece AS7825X and the sapphire tip AS7073X (1.0 x 14 mm). This is a particular dental laser device where the optical resonator is located very close to the handpiece, in this way avoiding the problems related to the delivery system of the laser beam and mainly due to the great absorption of this wavelength by water. The irradiation was performed with one passage of the beam on enamel in "lawn cut" way, under an "air/water spray".

Remineralization measurement by Raman Spectroscopy

Raman Spectroscopy has gained attention for the biomedical application due to its ability to provide a quantitative and qualitative chemical analysis of the biological sample [22].

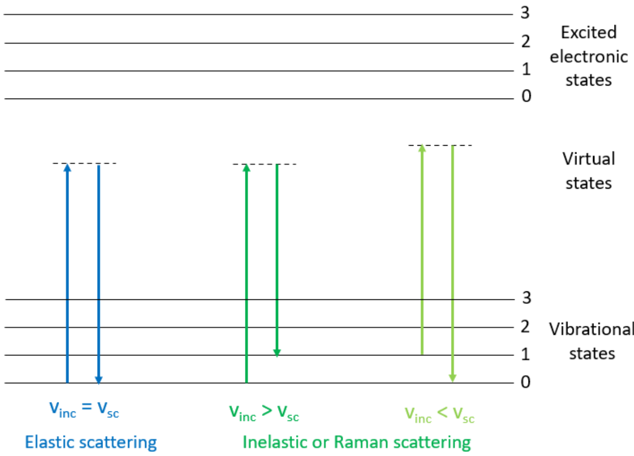

Fig. 2. Schematics of the Raman Scattering

The working principle of Raman Spectroscopy is based on the inelastic scattering of light in combination with a vibrational excitation of the molecular structure. Inelastic scattering occurs in rare cases when the molecule excited by the incident light returns one vibrational state lower or higher than its original state (see Fig. 2). This results in a frequency difference between the incident and scattered light, called Raman shift, that is specific to each molecule. Therefore, the position of the Raman peak can be used to discriminate the type of molecule present in the sample and its intensity indicates the concentration of the molecule [22].

Figure 3 (a) illustrates the schematics of the Raman spectrum of the tooth enamel [23]. As can be seen, there are several Raman peaks in the range from 550 to 1150 cm^{-1}, but the most prominent one is a peak around 960 cm^{-1} associated with the phosphate molecule ions (PO_4^{2-}). Silveira *et al.*, have used Raman Spectroscopy to measure the demineralization and remineralization of the enamel due to the application of the whitening product and saliva respectively [24]. They have shown that the intensity of the 960 cm^{-1} Raman peak decreases when the teeth are demineralized and increases with the remineralization process. In this work, the degree of enamel remineralization due to the application of three types of treatment (fluoride, laser, laser + fluoride) are going to be measured by the 960 cm^{-1} Raman peak's intensity. The teeth samples have been measured by HORIBA LabRam HR Evolution spectrometer with 785 nm wavelength at 5 different points each. The HORIBA software has been used to remove the background of the Raman spectra. The average of the five obtained spectra has been found for each tooth and the average of all teeth inside the group has been calculated. The intensity of the Raman peak at 960 cm^{-1} has been compared in the final representative spectrum of each group.

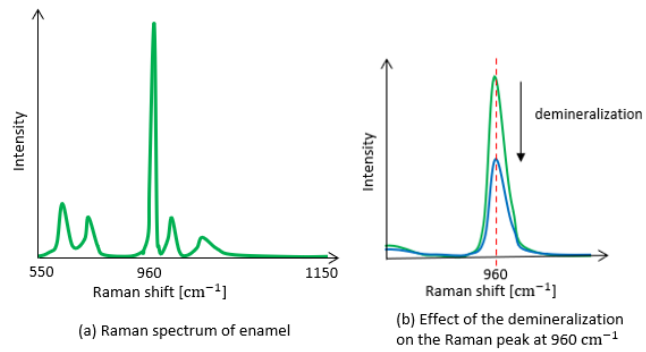

Fig. 3. Schematics of the Raman spectra of enamel

Results

Figure 4 illustrates the results of the experiment: an average of the Raman spectra of each group of teeth. As can be seen, all spectra correspond to the Raman spectrum of enamel found in the literature (Fig. 3, a) and have the highest peak around 960 cm^{-1}. The magnification of this region shows that the intensity of the peak increases with respect to the control only for the teeth treated by fluoride, which proves the remineralization abilities of this method. However, laser irradiation alone and in combination with fluoride resulted in the reduction of the peak's intensity. The difference between the control group and laser-irradiated teeth is very small, so it could be assumed that the laser has no effect on the teeth remineralization. However, a considerable difference between the fluoride treatment alone and with an application of laser implies that the laser has an influence on the peak's decrease. One of the explanations for such behaviour can be the morphological changes that the laser is known to introduce to the teeth. Thus, the microscopic image of the teeth from groups 3 and 4 shown in Fig. 5., illustrates the difference in the teeth structure of teeth treated by the fluoride and laser or by fluoride alone. It can be also seen that the focus on the right image is worse, which can significantly decrease the Raman signal intensity.

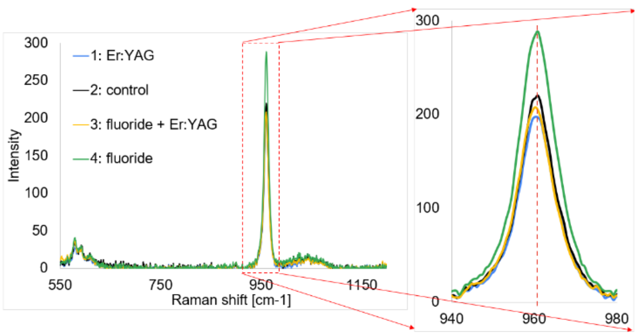

Fig. 4. Average Raman spectra of each of the four groups of teeth

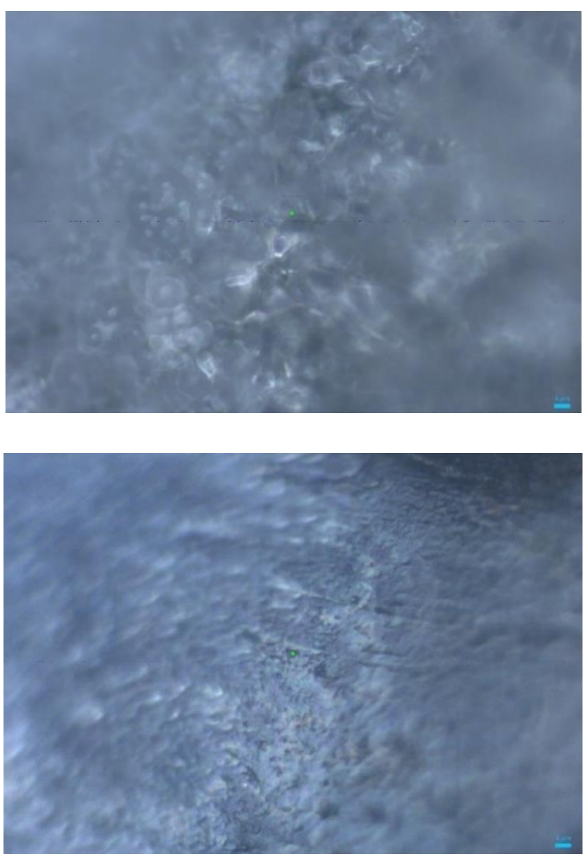

Fig. 5. Microscopic images of the sample teeth from the group 3 (left) and 4 (right)

Fig. 6 shows that the variation of the data from the teeth in the same group is considerable, which makes it difficult to differentiate between groups 1, 2, and 3. To the best of our knowledge, there is only one paper that has evaluated the effect of the laser treatment of the teeth using Raman Spectroscopy [25]. Kerr *et al.*, have applied Microwave energy to the teeth followed by the treatment with remineralization protocol. The results are not consistent: two out of four teeth are characterized by an expected increase of the Raman peak's intensity, but the peak has decreased for the other two teeth. This and the challenges with a focus due to the changed morphology can indicate that the Raman Spectroscopy is not a perfect tool for the remineralization assessment after the laser application.

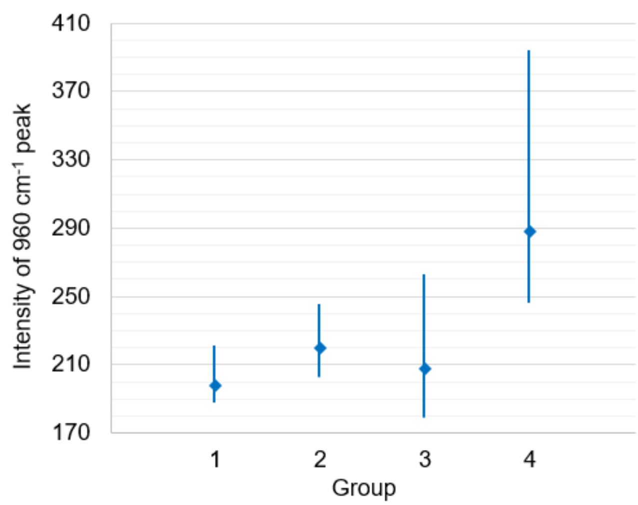

Fig. 6. Variation of the 960 cm^{-1} peak's intensity for all teeth

Discussion

Untreated dental caries is the most prevalent and common factor affecting health [26] and, on the global scale, it is estimated that they are prevalent among 2.3 billion adults with secondary dentition, and among 530 million children with deciduous dentition [27]. In 2015, the global cost of oral diseases was reported to exceed 540 billion dollars, consequently leading to major health and financial burden [28] and, for this reason, a great number of

research aiming to find new ways for decay prevention, has been performed in these last years.
Dental decay has a multi-factorial etiopathogenesis consisting in three main factors, the microorganisms (the dental plaque), the substrate (the fermentable carbohydrates in a diet), and the host susceptibility (the teeth and saliva) [29], and for each of them, several preventive approaches have been proposed. The two main methods suggested for obtaining a re-mineralization of tooth enamel are today fluoride and laser but opinions about their effectiveness are discordant. Xue and coll, comparing the effects of combined treatment of topical fluoride and semiconductor laser, concluded that lasers enhance the effect of fluoride on remineralizing but not on preventing enamel caries [30]. AlShamrani and coll. investigated the effects of combining erbium, chromium: Yttrium-scandium-gallium-garnet laser irradiation with fluoride application and concluded that acidulated phosphate fluoride application as well as laser irradiation prior to fluoride application increases enamel surface microhardness, so preventing the progression of enamel erosion [31]. Soltanimehr and coll. evaluated the efficacy of diode and CO_2 lasers along with calcium and fluoride-containing compounds for the remineralization of primary teeth and concluded that, while the highest microhardness was achieved after remineralization with CPP-ACP, the efficacy of the diode and CO_2 lasers was the same and no synergistic effect was found between materials and lasers [32]. Ceballos-Jiménez and coll. studied the acid resistance of dental enamel treated with remineralizing agents, Er:YAG laser and combined treatments and concluded that, although the combination of Er:YAG laser plus NaF and the single application of NaF showed values suggesting superior resistance to demineralization of dental enamel compared to all the other groups in the study, no statistically significant differences were found to support this assertion [33].
In our study, the conventional fluoride treatment method has shown expected results: the 960 cm^{-1} Raman peak's intensity has increased considerably showing the growth of the mineral content. The laser application for teeth remineralization has shown a questionable effect in different literature, which is also in agreement with our findings. Thus, the laser treatment has slightly decreased the Raman peak with respect to the control group, while the peak reduced significantly for the laser and fluoride treatment compared with the fluoride application only.
One of the possible explanations is that the morphological changes induced by the laser may complicate the focus of the Raman laser and hence deteriorate the overall signal intensity.

Conclusions

With the limits due to the limited number of samples, we may conclude that this preliminary study of the Raman Spectroscopy application for the evaluation of the remineralization effect of the Er:YAG laser treatment demonstrated a good effectiveness of fluoride application. Use of Er:YAG laser did not seem to achieve the expected remineralization of enamel, as well as the association laser and fluoride. Future work is aimed at evaluating a larger number of the samples and conducting the XRD measurement before and after treatment for the monitoring of morphological changes.

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