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ORIGINAL ARTICLE

The Effect of Er:YAG Laser and Diode Laser in Combination with MI Varnish on the Depth of Remineralization of Incipient Carious Lesions - An *In vitro* Study

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Abstract

Background: Dental caries is a multifactorial disease that results from an imbalance between pathological and protective factors. The imbalance produced favors demineralization of enamel. If detected early, non-cavitated lesions can be reverted with the help of remineralizing agents. The role of calcium-phosphate remineralization systems and the Er:YAG and diode lasers in the treatment of these lesions has recently been taken into account.

Aim: The aim of the study was to evaluate the remineralization potential of Erbium-doped yttrium aluminium garnet laser (Er:YAG laser), diode laser combined with MI varnish on the depth of remineralization of incipient carious lesions.

Methods: Forty eight human premolars were collected, conditioned with demineralizing agents to create artificial carious lesions. Then the samples were coated with nail paint leaving a uncoated window of 4 mm × 4 mm facially. The samples were then randomly divided into four groups (n=12): Group I –Control, Group II –MI Varnish, Group III – MI Varnish + Er:YAG laser, and Group IV –MI Varnish + Diode laser. For a period of seven days, specimens were conditioned with remineralizing agents followed by laser irradiation. Confocal laser scanning microscopy (CLSM) was used to assess the depth of demineralization and remineralization. Statistical Package SPSS 22.0 (SPSS Inc., Chicago, IL) was used to analyze the data. Kruskal–Wallis test was used to analyze the difference between the groups and post hoc Mann–Whitney test was used to compare the differences between the groups. The level of significance was set at $p < 0.05$.

Results: Samples in Group III showed greater remineralization depth (1488.235) compared to other groups with statistically significant ($p < 0.001$).

Conclusion: Within the limitations of this *in vitro* study, MI varnish alone is less effective compared to laser groups, MI varnish + Er:YAG and MI varnish + Diode laser which showed increased remineralization depths.

Keywords: Remineralizing agents, Er:YAG laser, Diode laser, MI varnish

Introduction

Dental caries is a biofilm-mediated, diet-modulated, complicated, and dynamic illness as it causes a net mineral loss in the tooth hard tissues. Scientific evidence indicates that the major factors are salivary dysfunction, fermentable carbohydrates, and cariogenic bacteria.[1]

Normal processes of demineralization and remineralization takes place in the oral cavity. Demineralization may be favoured due to changes in diet, oral hygiene, or microbial activity. The buffering effect of saliva, which enables calcium and phosphate ions to precipitate onto the tooth and produce new material favours remineralization. Hence, controlling the demineralization-remineralization balance is essential for preventing caries.[2,3]

Various remineralizing agents like fluorides, casein phosphopeptide amorphous calcium phosphate (CPP ACP), self-assembling peptides and herbal agents are accustomed to revert initial non-cavitated carious lesions.[3]

CPP-ACP is a nanocomplex generated from milk protein, casein, which aids in significantly raising the calcium and phosphate levels in the body. It increases the saliva's ability to buffer acids by steadily releasing calcium and phosphate, shielding teeth from acidic situations and encouraging remineralization. Low concentration and gradual release of calcium and phosphate to teeth are the main drawbacks of flourides.[3,4]

Fluoride varnish could be a standard remineralizing agent as it has prolonged contact time wuth the tooth surface acting as a slow releasing reservoir of fluoride. MI varnish is a 5% NaF (Sodium flouride) varnish containing CPP–ACP with sustained release of fluoride, calcium, and phosphate, thereby increasing the bioavailability of these minerals and promoting remineralization.[5]

Another recent development in remineralization is laser irradiation that decreases enamel permeability and solubility. Laser also inhibits the growth and multiplication of cariogenic bacteria, thereby acting as an adjunct in caries prevention. Both Erbium-doped yttrium aluminium garnet laser (Er:YAG laser) and diode laser are known to alter the morphological and chemical structure of enamel.[5,6]

Till date there is no study comparing the combined effects of MI varnish with Er:YAG laser and diode laser on its remineralizing potential of incipient carious lesions. Thus the aim of the study was to assess and compare the remineralization potential of Er:YAG laser, diode laser with MI varnish on incipient carious lesions.

Materials and Methods

This *in vitro* investigation was conducted in the Department of Conservative Dentistry and Endodontics for a period of six months. The Institutional Ethics Committee gave the study their seal of approval. Using G *power, version 3.0.1 (Franz Faul University, Kiel, Germany), sample size estimate was performed. With an effect size of 0.25 and a significance level of 0.05, 48 samples (12 in each group) would provide 80% power to identify significant changes.

For the study, forty-eight human premolar teeth free of cavities or cracks, that had been extracted for orthodontic reasons were chosen. Teeth were disinfected, cleaned with an ultrasonic scaler to get rid of the tissue scraps, and stored in distilled water until use.

Artificial caries like lesions were produced by storing the samples in 50 mL of demineralization solution for 48 hours at 37°C.

The demineralization solution consisted of:

2.2Mm $CaCl_2 \cdot 2H_2O$

2.2Mm $NaH_2PO_4 \cdot 7H_2O$

0.05M Lactic acid

After demineralization, samples were coated with nail paint leaving a 4 mm × 4 mm uncoated space facially (Figure 1). Samples were divided into four groups. Prior to remineralization, the depth of demineralization was examined using a confocal laser scanning microscope (CLSM) (Figure 2).

Figure 1: Premolars used for study

Group I: CONTROL

Samples which were not provided with any treatment.

Group II: MI VARNISH

Samples were conditioned with MI varnish using an applicator brush for four minutes according to manufacturer's instructions for seven days.

Group III: MI VARNISH + Er:YAG LASER

Samples were conditioned with MI varnish application for four minutes according to manufacturer's instructions for seven days followed by ER:YAG laser irradiation (Litetouch, wavelength 2940 nm; spot size 0.9 mm) and the laser was operated in a pulse mode at a distance of 5–7 mm perpendicular to the surface. The laser was used at 100 mJ and 10 Hz for five seconds with air and water cooling spray, and the average output power was 1 W.

Group IV: MI VARNISH + DIODE LASER

Samples were conditioned with MI varnish application with a brush for four minutes according to manufacturer's instructions for seven days, followed by diode laser irradiation (Wavelength 980 nm, Frequency 15 Hz). Laser was operated in a pulse mode at a distance of 5 mm (Power = 5W, Beam diameter = 600 µm, Pulse duration and Pulse interval = 30 ms, Power density = 62/5 W/cm2 and Energy = 150 J).

All the samples in each group received the remineralization treatment before being dried, placed in artificial saliva, and washed with deionized water. The same process was repeated for seven days, and then the samples were laser irradiated and submitted to CLSM to determine the depth of remineralization.

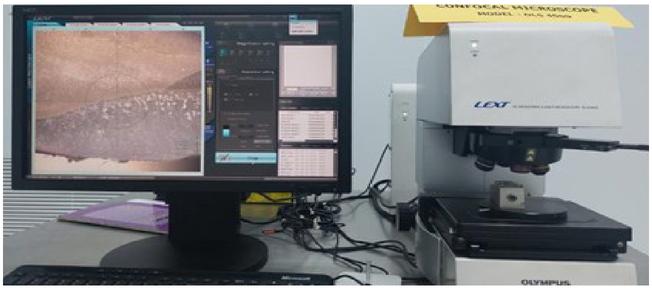


Figure 2: Confocal laser scanning microscope (Olympus, OLS4000)

Statistical analysis

Statistical evaluation G*power, version 3.0.1 (Franz Faul University, Kiel, Germany), created a power analysis. With an effect size of 0.25 and a significance level of 0.05, 48 samples (12 in each group) would have 80% power to detect significant differences. The statistical package SPSS 22.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data, and the threshold of significance was set at $p < 0.05$. After analyzing the degree of demineralization and remineralization among the groups using the Kruskal-Wallis test, the groups were compared using the post-hoc Mann-Whitney test.

Results

Table 1 and Figure 1 shows the data comparing the levels of demineralization in various categories. Higher median values were displayed by Group III (1669.71), Group II (1657.57), Group I (1626.53), and Group IV (1619.92). $p = 0.92$ indicated no statistical significant difference between the groups.

Table 2 and Figure 1 depicts the comparison of the levels of remineralization in various categories. The median values of the groups were- Group III (1488.23), Group IV (1334.50), Group II (1024.76), and Group I (706.97). Between the groups, there was a statistically significant difference ($p < 0.001$). To compare the demineralization and remineralization between the groups, the post hoc Mann-Whitney test was used. For demineralization, there was no statistically significant difference between the groups, but for remineralization, there was a statistically significant difference between all the groups ($p < 0.001$).

Table 1: Comparison of the demineralisation depth (in microns) among the groups using ANOVA

Groups	N	Minimum	Maximum	Mean	S.D	*p* value
Group 1 (Control)	12	1231.43	1879.98	1626.53	221.46	
Group 2 (MI varnish)	12	1245.67	1879.98	1657.57	219.31	0.922
Group 3 (MI varnish+Er:YAG)	12	1234.65	1876.98	1669.71	214.03	
Group 4 (MI varnish+Diode)	12	1243.34	1890.98	1619.92	206.00	

Table 2: Comparison of the remineralisation depth (in microns) among the groups using ANOVA

Groups	N	Minimum	Maximum	Mean	S.D	*p* value
Group 1	12	406.29	987.76	706.978	175.406	0.001*
Group 2	12	768.98	1249.09	1024.767	121.429	
Group 3	12	1234.65	1667.75	1488.235	123.251	
Group 4	12	1125.09	1567.43	1334.502	119.106	

*significant

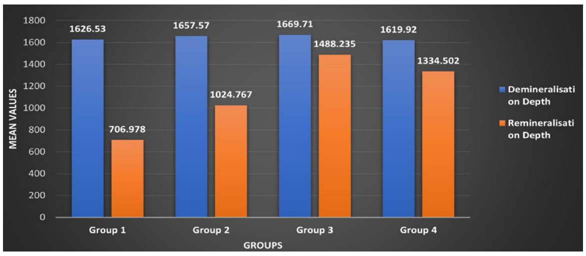
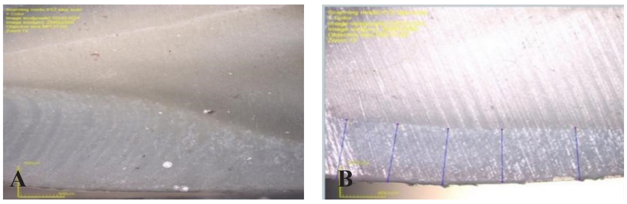
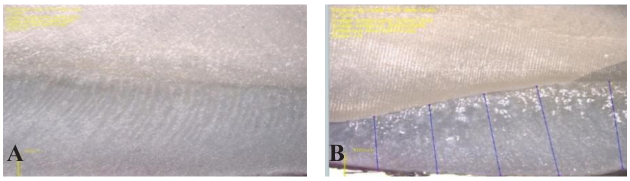


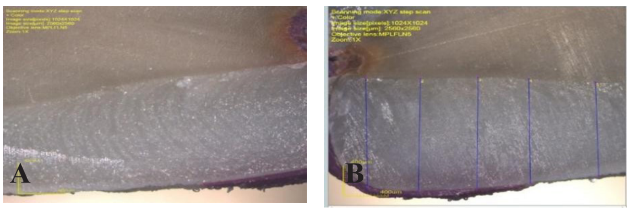
Figure 1: Comparison of the Demineralisation Depth and Remineralisation Depth (In Microns) among the groups



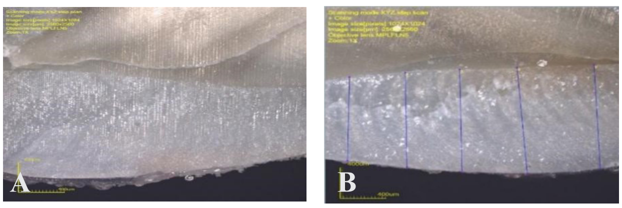
Group I



Group II



Group III



Group III

Figure 3: CLSM images of groups I, II, III, IV (A) demineralization and (B) remineralization (white area indicates demineralization, black area indicates remineralization)

Discussion

Caries management is time-sensitive and crucial because of its dynamic nature. Early detection frequently allows for reversal of the disease at its early stages, while later stages result in irreversibility. Enamel loses calcium and phosphorous ions as a result of demineralization. If the pH is adjusted and with availability of adequate amounts of calcium and phosphate ions to support remineralization, reversal may be possible.[7,8]

Topical application of remineralizing agents is one of the novel treatments being explored to slow or stop the growth of non-cavitated, demineralized lesions. These substances provide an environment that is favourable for enamel remineralization.[9]

The ability of the test agents to remineralize was examined using a confocal laser scanning microscope in this investigation. Nevertheless, CLSM revealed that specimens treated with MI varnish + Er:YAG Laser (Group III) had considerable deeper levels of remineralization compared to specimens in the other three groups (Figure 3).

MI varnish is a modification of CPP-ACP and it contains calcium, phosphate and fluoride ions. It increases contact time between the tooth and the agent and releases higher concentrations of bioavailable calcium, phosphate and fluoride. In the previous studies, application of MI varnish showed good results compared to other remineralizing agents.[10,2]

Laser is a minimal invasive approach for remineralization of tooth. The potency of light energy as a catalyst for remineralization has been extensively elucidated. Application of high intensity laser light leads to melting and recrystallization of enamel crystals.[11]

Laser irradiation causes alterations in enamel morphology by decreasing the water and carbonate content, increasing the pyrophosphate content, thereby decreasing the enamel solubility and enhancing its resistance to caries.[12,13]

Er:YAG laser along with MI varnish showed additive effect on remineralization. The results obtained in this study are in concurrence with the observations of Yassei *et al*. and Hossein Assarzadeh *et al*.[4] This might be possible because of the enhanced penetration of calcium phosphate of MI varnish when applied along with laser. The resultant morphological and permeability alterations in the enamel surface during laser application helps in enhanced penetration of calcium phosphate and increases the uptake of fluoride.

Diode laser along with MI varnish also shows additive effect on remineralization. It is similar to the results obtained by Zahra Bahrololoomi *et al*.[6] Diode laser is a small portable device with has many applications in dentistry and it is low in cost. Although diode laser radiation had increased the remineralization depth, there was no significant difference as compared to Er:YAG laser. This might be because the Er:YAG laser is specifically a hard tissue laser and causes increased melting of enamel crystals and increases fluoride uptake.

Further *in vitro* and *in vivo* studies are required regarding the parameters of lasers used, such as pulse energy, pulse duration, energy density, radiation time, and whether to use or not to use air or water for cooling during laser irradiation during the remineralization process.

Conclusion

Within the limitations of this *in vitro* study, MI varnish alone is less effective compared to laser groups, MI varnish + Er:YAG and MI varnish + Diode laser which showed increased remineralization depths. Considering the multifactorial and complex nature of the remineralization process, *in vivo* studies are required to better understand the clinical significance of the findings of this study.

Conflicts of Interest

Nil

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