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Effect of Two Remineralizing Agents on Artificial Demineralized Enamel Irradiated with Er, Cr:YSGG Laser: In-Vitro Study

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Abstract

Background: Dental caries is a widespread, noncommunicable disease affecting half of world's population. Thus, prevention rather than mere treatment of dental caries has become a decisive goal in dentistry. Therefor the adequacy of laser (Erbium, chromium-doped yttrium, scandium, gallium and garnet) irradiation and remineralizing agent were tested on artificially demineralized enamel.

Methodology: Twenty human molars were selected, scaled, polished and sectioned into two equal buccal and lingual sections (n=40). Surfaces were coated with acid resistance nail varnish except for enamel window of 4x4 mm. The specimens were embedded in demineralizing solution for 3 days. They were randomly divided into two groups (n=20) according to type of remineralizing agent, group (MI): MI paste plus and group (RE): Remin Pro. They were applied according to manufacturer's instructions. The groups were then divided into two subgroups (n=10); groups (MIL) and (REL). In both groups specimens were treated first with Er, Cr: YSGG Laser then remineralizing agents were applied. The weight % of Calcium and Phosphate were measured using EDX. The measurements were done before any treatment (control group), after demineralization and after remineralization. Representative specimens were selected for scanning.

Results: The results of the study showed that (MI, RE and MIL) weren't able to restore the minerals compared to the control group. While REL group was the only group similar to the control group. In addition, the intergroup comparison showed that REL had the highest remineralization compared to other groups.

Conclusions: The combined use of laser and remineralizing agents improved enamel remineralization

1 Introduction

The caries is the most widespread noncommunicable disease globally affecting half of world's population. [1] Approximately 3.9 billion people were affected by this oral condition, which exerted a substantial influence on individuals or communities regarding their health, and quality of life.[2] Thus, the prevention rather than conventional treatment of dental caries has become a decisive goal of modern dentistry.[3]

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The primitive state of caries are spot lesions, of a pearly appearance, White Spot Lesions (WSLs), which are caused due tosubsurface porosities. [4] The Prevalence of WSLs has been reported between 50 and 97%.[5] They are more common in subjects that lack a good oral hygiene or in those undergoing fixed orthodontic treatment.[6] The annulment of WSLs is an important part of prevention since it is a dynamic process with periods of demineralization and remineralization leading to apparent repair of the lesion.

There are many factors that might favor remineralization; the shape and structure of the tooth, quantity and quality of saliva, the composition of the bacterial biofilm, presence of fluoride, and the shift of biofilm PH.[7]

Topical fluorides such as sodium fluoride (NaF), acidulated phosphate fluoride (APF), and titanium tetrafluoride (TiF_4) are used as caries preventive methods.[8] Generally, fluoride act by replacing the OH- ion or any other impurities in hydroxyapatite crystal creating fluorapatite. Thus, the critical pH for demineralization (5.5) has to drop below the 4.5 to induce solubility of the fluoride enhanced tooth structure creating a resistant surface.

"Fluoride alone as remineralization strategy has not been sufficient to prevent dental caries". [9] As it leads to superficial remineralization leaving the deeper layers unaffected.

Subsequently, other remineralizing products have also been proposed in recent years such as casein phosphopeptide amorphous calcium phosphate (CPP-ACP) and Remin Pro®. [10, 11]

Casein phosphopeptide (CPP) is a milk-derived phosphoprotein that stabilizes high concentrations of calcium and phosphate ions in saliva with respect to the calcium phosphate tooth structure at acidic and basic pH. In the presence of fluoride ions, nanoclusters of CPP-stabilized ACP (CPP-ACP) or CPP-stabilized ACFP (CPP-ACFP) nanocomplexes are formed enhancing remineralization. [12, 13]

Remin Pro® which is a product that is recently released in the dental market it consists of fluoride, hydroxyapatite, and xylitol. it has a higher remineralization potential on enamel WSLs compared to MI paste, sodium fluoride and fluoride varnish, as per Bilgin et al. [12]

Alternative to the remineralizing agents, laser has been introduced as a possible mean for strengthening demineralized enamel. The most effective type of lasers for dental hard tissue applications are erbium family lasers. [13] The advantage of Er,Cr:YSGG over Er: YAG was explained due to its higher absorption by hydroxyapatite, which is the main component in enamel. Thus Er,Cr:YSGG shows deeper penetration of 21 micrometers in enamel, and 15 micrometers in dentine in comparison to Er:YAG laser wavelength that penetrates approximately seven micrometers in enamel, and five micrometers in dentine. Leading to greater cutting efficiency with little heat generation.[14] In addition, some studies have shown that Er,Cr:YSGG lasers can increase the acid resistance of demineralized enamel.[15] The coactive potential of the laser and remineralizing agents improves the management of caries, with apparent reduction in the solubility and permeability of enamel. [16]

In spite of the fistful research on caries inhibition, there is little evidence about the effects of Er, Cr:YSGG lasers, combinedwith remineralizing agent in demineralized enamel.[17] Therefore, this study aims to assess the mineral gain of artificially demineralized enamel after being treated withlaser and remineralizing agents. The Null Hypothesis is that there is no difference in remineralization when laseris used in conjugation with the remineralizing agents compared to the use of remineralizing agents alone.

2 Materials and Methods

This study was performed at the department of conservative dentistry, Faculty of dentistry, Modern Science and Arts University (protocol registration number ETH23)

2.1 Materials

Two remineralizing agents were used in this study. Minimal intervention paste, MI Paste Plus paste containing active ingredient (CPP-ACP) RECALDENT™ and Remin Pro Material's composition, manufacturer and batch no are represented in **(Table 1).**

Material	Manufacturer	Chemical composition	Batch No.	Site URL
Minimal intervention paste (MI Paste Plus)	GC Europe N.V Leuven, Belgium	CPP-ACPF* (Casein Phosphopeptide –Amorphous Calcium Phosphate Fluoride), Glycerol, D-Sorbitol, CMC Na, Propylene glycol, Silicon dioxide, Titanium dioxide, Xylitol, Phosphoric acid, Flavoring, Sodium saccharin, Ethyl p-hydroxybenzoate, Propyl p hydroxybenzoate, Butyl phydroxybenzoate. 900 ppm fluoride. With the PH of 7.8	9050416	https://www.gcamerica.com/products/preventive/MI_Paste/
Remin Pro	Voco GmbH Cuxhaven, Germany	Nano-Hydroxy apatite, sodium fluoride (1450 ppm fluoride) and xylitol	50002002	https://www.voco.dental/us/portaldata/1/resources/products/folders/us/remin-pro_fol_us.pdf

Table 1. Materials composition, manufacturer and batch no.

2.2 Methods

2.2.1 Sample selection

20 human molars were freshly collected from oral surgery clinic. Patients signed a consent form explaining the reason for teeth extraction and that their teeth will be used for research purposes. Teeth were selected on the basis of the following inclusion criteria: patients between 20-30 years old, intact buccal and lingual enamel surface, patients free from systemic disease and abnormal oral habits.

Extracted teeth were cleaned from debris and blood under running water, scaled, and polished with fluoride free fine pumice and soft rubber cups rotating at low speed under water coolant. The teeth were inspected using a magnifying lens to make sure they were free from any pathological abnormalities. The teeth were then stored in phosphate buffer saline containing 0.2% sodium azide for maximum period of one month.

2.2.2 Sample size and power

A power analysis was designed to have adequate power to apply a statistical test of the null hypothesis that there is no difference between laser, and the two remineralizing agents regarding the remineralization of enamel. By adopting an alpha (α) level of 0.05 (5%), a Beta (β) level of 0.20 (20%) i.e., power=80% and an effect size (f=0.801) calculated based on the results of Ahrari, Farzaneh, et al.; the predicted sample size (n) was a total of twenty-four samples. i.e., twelve samples per group and six samples per subgroup. Sample size calculation was performed using G*Power version 3.1.9.

2.2.3 Specimens preparation

Each tooth was embedded in plastic mold filled with self-curing acrylic resin. The teeth were sectioned using a microtome (Isomet 4000, USA) under water coolant into two equal buccal and lingual sections. The outer enamel surface of each half was polished using soflex discs attached to slow speed contra angle hand piece. To standardize the demineralized treatment area on each specimen a thermoplastic material (polycarpolactone) was softened in a hot water bath for two minutes, flattened into sheets then a window of four by four mm was cut. The buccal, lingual surfaces and pulp chamber were coated with acid resistance nail varnish except for treatment area of 4x4 mm.

2.2.4 Grouping of specimens

A total number of 40 specimens (20 buccal specimens and 20 lingual specimens) were randomly divided into two equal groups (n= 20) according to application of remineralizing agent used, group (MI): MI paste plus, and group (RE): Remin Pro. In these groups, the remineralizing agents were applied according to manufacturer's instructions. Each group was then divided into two subgroups according to the method of application (n=10). Group (MIL): specimens were treated first with Erbium, chromium-doped yttrium, scandium, gallium and garnet (Er,Cr:YSGG) Laser then MI paste plus was applied. Group (REL): specimens were treated first with erbium, chromium-doped yttrium, scandium, gallium and garnet (Er,Cr:YSGG) Laser then Remin Pro remineralizing agent was applied.

2.2.5 Assignment of intervention

The randomization process was made using computer software www.random.org. The randomized numbers were written on a sequentially numbered opaque sealed envelope. This was followed by Allocation Concealment and Implementation.

2.2.6 Measuring mineral content

The mineral content was measured three times; before being subjected to any treatment (control group), after demineralization and after remineralization.

After each period the wt% of Calcium and Phosphate were measured using Energy Dispersive X-ray Spectroscopy (EDX).

2.2.7 Preparation of demineralizing solution

Artificial white spot lesion was done by placing the specimens in 10 ml of freshly prepared demineralizing solutions. The demineralizing solution was prepared according to Rafiei et al, 2020.[18] 0.05M lactic acid, 2.2 mm calcium chloride ($CaCl_2$), 2.2 mm sodium dihydrogen phosphate (NaH_2PO_4), and 0.2 ppm fluoride were added together and mixed withsaline. The mixture was then placed on a magnetic stirrer to ensure proper mixing. The PH of the mixturewas adjusted with 50% NaOH to reach pH 4.5 using a PH meter. The solution was replaced on a daily basis. The Specimens were immersed for three days as per Weir et al, 2012.[19]

2.2.8 Surface treatment of demineralized specimens

According to literature a conflict existed regarding the application of laser treatment prior to or after application of remineralizing agents. Some studies showed that laser causes structural changes preventing proper remineralizing agent penetration[20] while others believed that laser enhanced the action of remineralizing. Moreover, the parameters selected in this study for laser application enhanced etchingrather than sealing according to serdar-eymirli et al. 2018. [21] Therefore, a pilot study was performed to detect the effect of laser before or after remineralizing agent application.

The pilot study showed that application of laser before remineralizing agents gave better results.

2.2.8.1 Surface treatment by remineralizing agent with or without laser

In both groups (MI) and (RE) the remineralizing agents were added on the treatment windows for five minutes, with the help of brush applicator tip in rubbing technique. The specimens were then rinsed and stored in distilled water for 24hrs. In groups (MIL) and (REL) (Er : Cr YSGG) laser (Waterlase MD turbo, Biolase,CA, USA) was used following parameters (single pulse) with a wavelength of (2.780nm), 2mm spot diameter , 0.25 W power setting and irradiation duration of ten seconds. [21]

To promote a uniform irradiation, the tip was positioned perpendicular to the enamel surface, moving in circular motion to make sure that the whole demineralized surface of 4mm was treated by laser since the spot diameter was 2mm, and the irradiation was performed manually in one direction with a consistent motion.

The mineral content (% Ca, ph and Ca/p ratio) of all treated samples were measured after 24 hour of distilled water storage using Energy Dispersive X-ray Spectroscopy.

2.2.9 Scanning electron microscope

For each group, representative specimens were selected for microscopic analysis. This was done to determine the structural changes on enamel surface after treatment using Scanning electron microscope (SEM) with EDX analysis unit. At magnification 1000x. Specimens were first polished using hand pressure on stationary wet silicon carbide abrasive paper (MicroCut8, Buehler, Lake Bluff, IL, USA) of 800 grit.

2.2.10 Statistical analysis

Numerical data were presented as mean and standard deviation (SD) values. They wereexplored for normality by checking the data distribution, and using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data showed parametric distribution so one-way ANOVA followed by Tukey's post hoc test was used for intergroup comparisons and repeated measures ANOVA. Bonferroni post hoc test was used for intragroup comparisons. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with was performed with IBM® SPSS® Statistics Version 26for Windows.

3 Results

3.1 In the Intragroup comparison for Calcium

After remineralization treatment, none of the remineralizing agents were able to restore the Ca content of the tooth structure to its pre-demineralization levels except for REL. In comparison to the other treatment groups, REL showed the highest Ca regain (35.11± 3.70). While in MI, MIL and RE the mineral content for Ca was (29.01, 35.23 and 30.09) respectively which was significantly less than the baseline for each group (40.26±1.12) for MI, (44.31±2.86) for MIL, (35.62±1.16) for RE and (37.29±3.48) for REL as shown in.

Interval	Weight (%) (Mean±SD)			p-value
	Control	Demineralization	Treatment	
MI	40.26±1.12^{A}	31.27±0.50^{B}	29.01±3.01^{B}	<0.001*
MI+laser	44.31±2.86^{A}	34.56±3.01^{B}	35.23±1.40^{B}	<0.001*
Remin pro	35.62±1.16^{A}	31.84±0.39^{B}	30.09±1.92^{B}	<0.001*
Remin pro+ Laser	37.29±3.48^{A}	32.88±1.08^{A}	35.11±3.70^{A}	0.065ns

(Table 2)

Table 2. Mean, Standard deviation (SD) values of weight percentage (%) for different groups regarding intragroup comparison for calcium content

** Different superscript letters indicate a statistically significant difference within the same horizontal row *; significant (p ≤ 0.05) ns; non-significant (p>0.05)*

3.2 Intragroup comparison for Phosphate

In all treatment groups, the control group showed the highest phosphate concentration which decreased significantly in all groups after demineralization with the mean value (15.45±0.14, 15.45±0.5, 15.27±0.31, 15.39±0.19). After remineralization treatment, none of the remineralizing agents (alone or in combination with laser treatment) were able to restore the phosphate content to tooth structure compared to control group. The mean and standard deviation for MI, RE, MIL and REL were (13.75±1.19, 14.24±0.79, 15.66±0.46 and 15.62±0.61) respectively that were significantly lower to each control group of each treatment (18.06±0.42) for MI, for (17.88±0.56) for MIL, (17.82±0.27) for RE and (17.92±0.30) for REL. **(Table 3)**

Interval	Weight (%) (Mean±SD)			p-value
	Control	Demineralization	Treatment	
MI	18.06±0.42^{A}	15.45±0.14^{B}	13.75±1.19^{C}	<0.001*
MI+laser	17.88±0.56^{A}	15.45±0.59^{B}	15.66±0.46^{B}	<0.001*
Remin pro	17.82±0.27^{A}	15.27±0.31^{B}	14.24±0.79^{C}	<0.001*
Remin pro+ Laser	17.92±0.30^{A}	15.39±0.19^{B}	15.62±0.61^{B}	<0.001*

Table 3. Mean, Standard deviation (SD) values of weight percentage (%) for different groups regarding intragroup comparison for phosphate content

** Different superscript letters indicate a statistically significant difference within the same horizontal row *; significant (p ≤ 0.05) ns; non-significant (p>0.05)*

3.3 Intragroup comparison for Ca/P

In comparing the mean values of Ca/P content of the control group to that after remineralization within each study group no statistical significance was found except for MIL group. It showed significant decrease in the Ca/P content after treatment. Meanwhile, the means and standard deviation of each treatment group compared to its control are; MI/C (2.13±0.30/2.23±0.10), RE/C (2.12±0.19/2.00±0.07), MIL/C; (2.25±0.10/ 2.48±0.16) and REL/C; (2.25±0.24/2.08±0.21) respectively shown in **(Table 4).**

Interval	Weight (%) (Mean±SD)			p-value
	Control	Demineralization	Treatment	
MI	2.23 ± 0.10^{A}	2.02 ± 0.02^{A}	2.13 ± 0.30^{A}	0.106ns
MI+laser	2.48 ± 0.16^{A}	2.24 ± 0.18^{AB}	2.25 ± 0.10^{B}	0.011*
Remin pro	2.00 ± 0.07^{A}	2.09 ± 0.05^{A}	2.12 ± 0.19^{A}	0.125ns
Remin pro+ Laser	2.08 ± 0.21^{A}	2.14 ± 0.07^{A}	2.25 ± 0.24^{A}	0.245ns

Table 4. Mean, Standard deviation (SD) values of weight percentage (%) for different groups regarding intragroup comparison for calcium phosphate content
**Different superscript letters indicate a statistically significant difference within the same horizontal row *; significant ($p \le 0.05$) ns; non-significant ($p>0.05$)*

3.4 Scanning Electron Microscope (SEM) examination of enamel surface at different treatments

SEM of specimens treated by MI paste plus & RE showed reduced enamel micro porosities with different sized granules scattered on the surface of remineralized enamel together with remnants of remineralizing agent. However, the specimenstreated with RE agent showed a more uniform covering and granular distribution of minerals. **(Figure1 & Figure2).** While the SEM of specimens treated with laser in combination with remineralizing agents showed smoother surface enamel and more obvious appearance of calcium phosphate in the form of adherent globular layers sealing the enamel micropores giving lava flow appearance **(Figure3).**

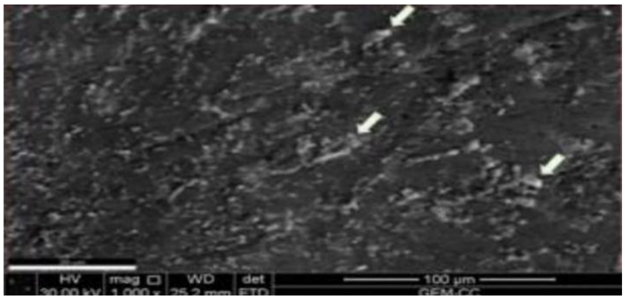

Figure 1. SEM of the representative specimens after MI Paste application.

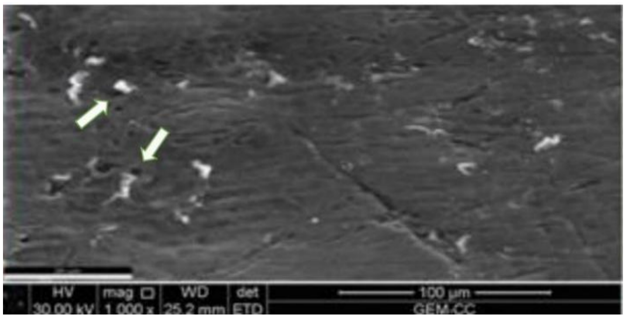

Figure 2. SEM of the representative specimens after Remin Pro application.

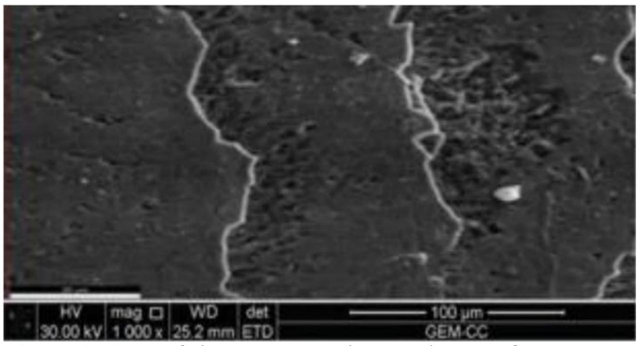

Figure 3. SEM of the representative specimens of REL& MEL groups.

4 Discussion

Fluoride is the gold standard in remineralization, however its sole use as remineralization strategy lacks to prevent dental caries at the population level. [22]

Its topical effect depends on its constant presence in the oral cavity (patient compliance) and also rely on the patient's oral hygiene. In addition to its improper effectiveness in preventing caries on the occlusal surface. [23] Therefore, other remineralizing agents have been proposed as CPP-ACP and nano-hydroxyapetite.

As of late the use of CPP-ACP has been increasing as a tool for prevention and treatment of enamel caries. MI Paste Plus contains CPP-ACP and 900 ppm fluoride. It is reported that the addition of fluoride into CPP-ACP amplifies its remineralization ability on initial enamel lesions in contrast with either CPP-ACP or sodium fluoride alone. [24, 25]

Recently introduced in the dental market is Remin Pro® which is a water borne cream that is made of, Nano hydroxyapatite, xylitol and 1450 ppm of fluoride. It is assumed that these ingredients increase the insertion of hydroxyapatite and fluoride into enamel lesions. Moreover, it limits the adhesion of bacterial plaque to enamel. [26]

Laser irradiation of the enamel has also been proposed for prevention of caries. Due to the fact that when enamel matrix is irradiated the surface anatomy changesmaking it less permeable thus inhibiting acid diffusion, and there by reduces enamel demineralization. The erbium lasers used for this purpose are erbium: yttrium–aluminum–garnet (Er:YAG) laser and erbium and chromium:yttrium – scandiumgallium - garnet (Er,Cr:YSGG) laser. [27]

Er, Cr: YSGG was selected in this study as it exhibits the highest absorption of all infrared lasers in water and hydroxyapatite, and are thus ideally suited for different dental procedures. The significant synergism of laser irradiation combined with topical remineralizing agents particularly fluoride in increasing enamel resistance has been reviewed by Ana et al. (2012)[19] It was proposed that laser irradiation aid in the formation of microscapes in enamel which can improve the following uptake of remineralizing agent.

However fewer studies have evaluated the use oflaser with other remineralizing agents. Therefore, this study was performed to determine the efficiency of remineralizing initial demineralized enamel, either through the sole use of remineralizing agents (Minimal intervention paste and Remin pro paste) or in combination with laser.

The results of this study showed that most of the treatment groups (MI, RE and MIL) weren't able to restore the calcium and phosphate compared to the control group where the specimens were not subjected to any type of treatment (demineralization and/ or remineralization). REL group was the only group that was statistically similar to the control group regarding the regain of the calcium content. These results were in accordance with Elahe Rafiei et al., Kamath U et al., and Heravi et al.[18, 20, 28] They showed that laser irradiation before application of Remin Pro is more effective in the deep layers of enamel than individual laser therapy. In addition, thelaser irradiation made effectiveness possible by facilitating Remin pro penetration into the depths of the sample. However, these results were in contradiction with Nastaran et al.[27] which showed that when laser was used with a fluoride containing remineralizing agent was not effective. This might be related to the use of different type of teeth (deciduous teeth) compared the use of permanentteeth in the present study.

When the treatment groups were compared to each other, it was found that when laser was applied first followed by the remineralizing agents, it enhanced the Ca and phosphate content compared to the application of remineralizing agents alone either MI or RE. Different studies have also reported increase in remineralization efficiency when remineralizing agent was applied on laser irradiated enamel.[27, 29]

In the present study Er, Cr: YSGG laser with the parameters of 0.25 W and 20 Hz were used. The used parameters were commensurate with the previously reported studies.[22, 23, 30] Oliveria et al. [24] used different parameters of the laser (power and frequency 20–50Hz)and found decreased microhardness with 0.75 W but increased microhardness with 0.25 W, 0.50 W. furthermore, even though some authors recommended the use of water spray to prevent any side effects of laser irradiation, no water spray was used in this study, with accordance to Hossain at al. and Samar at al. [8,31] Both found that the depth of the lesions produced after Er, Cr:YSGG irradiation with water spray were significantly greater than those without water spray deducing that water played an important role in ablation of tissues.

The synergistic effect of laser with fluoride in prevention of demineralization has different mechanisms. Firstly, new crystalline phases were formed when remineralizing agents were applied after using Er, Cr: YSGG laser treatment that are larger than the initial ones as was observed in an X-ray diffraction study by Zezell et al. [25] These new crystals, with the fluoride present could enhance the subsurface calcium and phosphate content due to increased uptake of these ions by laser. Add to that, the increased resistance to demineralization could be accredited to the better retention of calcium and phosphate ions for a longer time after laser irradiation due to better penetration of these ions deep into the micro spaces formed by the recrystallization of the enamel crystals by laser as well as the heat generated in depth by laser. On top of that the increased enamel resistance to demineralization is due to the formation of a significant increment of CaF2 and retention in the irradiated samples.[25] SEM analysis of this study showed globular deposits on enamel surface in the specimnes treated with MI or Remin pro **(Figure3)** which is suggested by some studies to be CaF crstals plus laser group. When the specimens were treated with laser plus remineralizing agent, amorphus fluorapatite crystals maybe formed producing asmoother, more homogenous glazed surface according to Aminabadi et al. [26] This was seen in the SEM photomicrographs of the present study in groups MIL and REL **(Figure3).** The findings of this study may indicate that in patients suffering from WSLs or incipient caries the combined use of remineralizing agents with laser can be efficient.

Moreover, the formation of CaF2-like layers over the tooth surface may explain why EDX revealed absence of fluoride in all groups.[32] Another explanation by Mohan et al. [33] is that when enamel was irradiated by laser it enhances fluoride uptake into the crystalline structure of the enamel in the form of firmly bound fluoride that cannot be detected by fluoride.

The non-significant difference in the Ca/P ratio between the treated specimens (MI, MIL, RE and REL) maybe related to the demineralization of specimens and laser parameters. According to Serdar-Eymirli et al. [21] the laser parameters used in this study enhanced more depth in the microporosities created after demineralization treatment.

Moreover, the specimens were demineralized by keeping them in demineralizing solution (CaCl2, NaH2 PO4, Lactic acid and Fluoride) for 72 hours at 37° C. The concentration of both calcium and phosphates, in the solution causes the dissolution of tooth structure Ca^{2+} and PO^{42-} ions only at the enamel subsurface leaving an intact outer enamel surface thus simulating an early enamel lesion. As tooth calcium and phosphate ions diffuse outwards and in the presence of fluoride already present in demineralizing solution remineralization at the surface becomes more and more likely where the mineral content is higher than the body of the lesion. This might have hindered the penetration of remineralizing agent.[34]

The results of this study highlight those different types of remineralizing agents have different interactions with laser and subsequently different effect on tooth structure. Further investigations are required to evaluate the acid resistance of the remineralized laser treated enamel at different pH and with different laser parameters. The null hypothesis of this study was rejected as there is apparent difference in the use of remineralizing agents alone or combined with laser

Limitations

This study has a number of limitations. Firstly, the human oral environment was not imitated and the assessment was only of short-term effects of the remineralization agents that were studied. For this reason, long-term evaluation of the most effective agents in situ or in vivo might be recommended for prospective studies.

Secondly the lack of comparison of different laser parameters may be regarded as a limitation of the present study. Finally, a general limitation for laser treatment clinically is that irradiation of the inaccessible areas such as proximal tooth surfaces is still a challenge. Added to that the irradiation was performed as hand irradiation and not by the use of a motor- driven computer-controlled stage.

Further investigations are required to determine the efficiency of combined treatment of laser and remineralizing agents to resist acid demineralization. In addition to testing the effect of different laser parameters and different types of remineralizing agents available in the dental market on the remineralization efficiency on tooth structure.

5 Conclusions

Within the limitation of this study the following conclusions maybe drawn; the combined use of laser and remineralizing agents improved enamel remineralization with particular reference to Remin pro compared to MI paste. The sole use of remineralizing agents alone might provide less remineralization effect than when used with laser.

Conflict of interest

The authors have declared no conflict of interest.

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