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Remineralizing Effect of Diode Laser and Amelogenin Peptide on Early Enamel Carious Lesion

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ABSTRACT

Purpose: This study was done to investigate the efficacy of Diode laser and Amelogenin peptide on the remineralization of early enamel carious lesions. **Materials and methods:** Artificial carious lesions were created on the buccal and lingual surfaces of 60 enamel specimens. Specimens were assigned to three groups of 20 specimens per each according to the remineralizing protocol: G1 (Diode laser), G2 (Diode laser + Amelogenin peptide), G3 (Amelogenin peptide). The groups were further randomly subdivided into two groups (n=10) whether specimens subjected to static remineralization or pH cycling. Surface micro-hardness was assessed at baseline, demineralization and after treatment. Surface microhardness values were analyzed using one way ANOVA and Tukey's post-hoc test. **Results:** Surface microhardness values at static remineralization were; G1=G2>G3. No statistically significant difference was found between the three groups after pH cycling. **Conclusion:** Diode laser enhanced remineralization of incipient carious lesions compared to amelogenin peptides.

KEYWORDS

Enamel remineralization, Diode laser, Amelogenin peptide

INTRODUCTION

Nowadays the Minimal Invasive Dentistry (MID) concepts are widely used for treating dental caries[(1)]. The MID depends on prevention and early intervention[(2,1)]. It aims to minimize time loss and patient discomfort [(2)]. It relies basically on the remineralization of incipient carious lesions [(3)], advocating the biological and therapeutic approaches[(4)].

There are several types of remineralizing agents such as fluorides[(5)], Casein phospho peptides (Cpp)[(6)], calcuim sodium phosphosilicate

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(bioactive glass)[6], nanohydroxyappetite (carbonate hydroxyapatite nanocrystals) [5]. These remineralizing agents have proven efficacy in remineralziation of incipient carious lesions with variable degrees[6,7].

Recently tissue engineering field begins to be exploited in MID through using self assembly peptides.Which are biomimetic materials that aim to be used as scaffold for tissue regeneration and can be used for treatment/prevention of dental caries[8]. Amelogenin is claimed to be a predominant enamel matrix protein which is essential for enamel biomineralization and act as key roles in regulating nucleation, growth, morphology and organization of developing enamel mineral phases[9]. The unique hydrophilic functional domain structure of amelogenin is responsible for initializing hydroxyapatite nucleation and promoting biomimetic remineralization of enamel because it contains acidic and basic residues that self assembly and promote the adsorption of calcium and phosphate [10].Which forms initially in developing enamel as linear arrays and subsequently transforms into apatite crystals [11].

Parallel to the track of material innovations, different surface pretreatments were also advocated to enhance the surface remineralization; among these phosphoric acid pretreatment and microabrasion. Recently lasers are gaining wider acceptance as physical surface modifying tool to enhance surface properties of the demineralized incipient carious lesions making them more resistance to any further acidic attacks [12].

A previous study proposed some hypotheses discussing the acid resistance effect induced by lasers. First; induction of melted surfaces and crater like holes by the co_2 and Nd:YAG Lasers. Second improving the crystallinity after Er:YAG ablation. Third reduction in the enamel solubility without sever enamel alteration induced Er:YAG and Er;cr:YSGG lasers [13].

On contrary previous study found that laser didn't enhance the remineralization of incipient carious lesion [14]. However, the impact of combination between lasers and biomimetic remineralizing agent wasn't tackled in literature. Thus this study was carried out to elaborate the effect of the combination between lasers and the biomimetic amelogenin on remineralization of enamel. The null hypothesis tested: there is no difference in the remineralization induced by lasers, amelogenins and combination of both in both static and pH cycling conditions.

MATERIALS AND METHODS

Research ethics committee approval (REC) was obtained from Faculty of Dental Medicine for Girls Al-Azhar University at(Ethics code REC-OP-21-07). A total of thirty non- carious human premolars extracted for orthodontic reasons (age range 11-14 years) were used in this study. The teeth were thoroughly cleaned of its debris, calculus, and soft tissues using a low speed hand piece with pumice paste. The specimens were stored in distilled water for a month till the time of the study [15]. All teeth were obtained from patients who have already signed consent form mentioning their approval of using their teeth.

Peptide synthesis

It was synthesized by Phoneix Pharmaceuticals. U.K in the form of powder which consists of sequence of amino acid (Leu-Pro-Pro-His-Pro-Gly-His-Pro-Gly-Tyr-Ile). In according to a previous study the peptide was dissolved in 100ml of distilled water and stored at 4°C.Then the peptide was added to a remineralizing fluoridated medium which consists of (1.5mm $CaCl_2$, 0.9mm NaH_2PO_4, 0.15m Kcl at PH 7.0 and 2ppm NaF was added). The solution was centerfugied (10.900 x g,4°C,20min) before use [16].

Specimens preparation

The crowns of the premolars were separated from the roots and cut vertically mesiodistally to obtain buccal and lingual halves using a diamond coated saw with continuous water cooling [16]. Specimens were embedded in acrylic resin blocks exposing their buccal or lingual surfaces facing upwards.

After setting of the acrylic resin the specimens were polished using finishing and polishing disks (Sof-lex Pop-On Disks 3M ESPE, St Paul,MN,USA) with low-speed hand piece and micro motor. Each specimen was numbered at the bottom of the block. On the facial surface of each specimen a window was done by using a ruler and marked by a marker; subsequently an acid resistant nail polish (Yolo,France) was applied to the whole surface leaving the demarcated window of exposed enamel (6mm x 4mm).

Artificial enamel carious lesion was created by immersing the specimens in a demineralizing solution. The solution contained 2.2 mM $CaCl_2$ (Calcuim Chloride), 2.2 mM NaH_2PO4 (Sodium dihydrogen orthophosphate dehydrate), 0.05 mM acetic acid; the pH was adjusted with 1 M KOH (Potassium Hydroxyide) to 4.4. Each specimen was immersed separately in a daily renewed demineralizing solution for 4 days until a uniform white spot lesion was created [17].

The sixty specimens were randomly divided into three groups using online randomization software into groups of 20 specimens. Group one: diode laser (Picasso, made in Germany)was applied with wavelength 810nm 2 watts for 90 seconds in a continuous non contact mode at a constant distance 2 mm. The sample was set on an articulator by glue and then the distance between the sample and the pin of the articulator was measured and the tip of the laser was placed beside the pin, ***figure 1***. The handpiece of the laser was moving uniformly and longitudinally over the marked window [18].

Group two diode laser was applied as it was applied for the first group. Each sample in group two was immersed separately in 1.5 ml volume of the peptide solution in the shaker incubator at temperature 37°C for 12 days. Specimens were placed with their facial surface embedded in the solution; with subsequent replacement of solution every two days. The peptide solution was stored at 4°C. The specimens were immersed in beakers separately and placed in a tightly closed box to avoid evaporation of the solution.

Group three Amelogenin peptide was applied as it was applied as in group two without laser treatment. The groups were further subdivided into two groups of 10 specimens per each according to static remineralization and ph cycling.

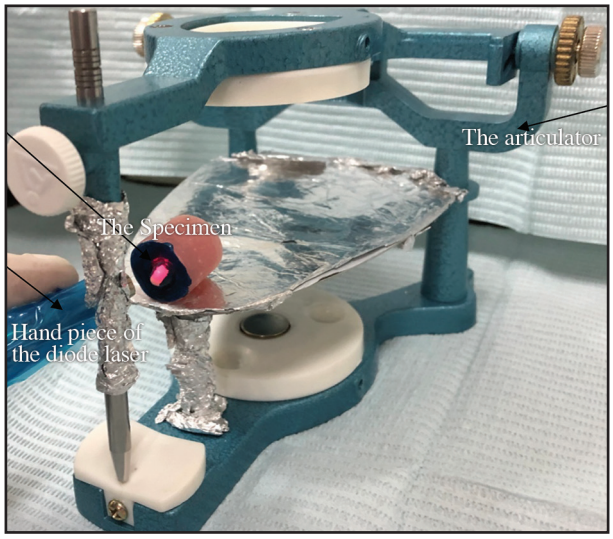

Figure (1) Application of diode laser

Static remineralization

The specimens were immersed separately in a 5ml daily renewed artificial saliva (1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.15 M KCl at PH 7.0) for 14 days [17].

PH Cycling

The specimens were immersed separately in a demineralizing solution (DES) the same solution used for creating the artificial carious lesion for 6 hours, followed by rinsing with distilled water and immersion in the same quantity of remineralizing solution (RES) for 18 hours. This procedure was carried out at 37c°. The RES and DES solutions were substituted daily, and the cycles were repeated for 14 days. After 5 days, the samples were immersed in a remineralizing solution for 2 days [19].

Microhardness assessment, (figure 2):

All the samples were subjected to microhardness assessment three times. At base line, after demineralization and after surface treatment. Microhardness of the specimens were determined using digital Display Vickers Microhardness Tester (Model HVS-50, Laizhou Huayin Testing Insturment Co.,Ltd China) with a Vickers diamond indenter and a 20X objective lens. A load of 100g was applied to the surface of the samples for 15 sec. Three indentations which were equally placed over a circle and not closer than 0.5 mm to the adjacent indentations, were made on the surface of each sample. The diagonals length of the indentations were measured by built in scaled microscope and Vickers values were converted into microhardness values. Mean of the three readings were calculated to obtain one reading once at the time.

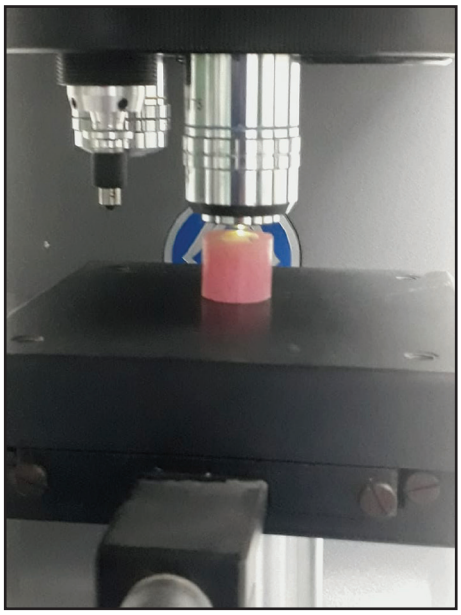

Figure (2) Testing the specimen's microhardness

Micro-hardness was obtained using the following equation:

HV is Vickers hardness in Kgf/ mm^2 $HV= 1.854P/d^2$

P is the load in Kgf and

d is the length of the diagonals in mm

Comparisons between groups was done using One way analysis of variance (ANOVA) test, followed by a Post hoc test when showed significant. Comparison between static and ph cycling remineralization protocols was done using student's t test for all treated groups.

RESULTS

The results of the one way ANOVA analysis for the effect of different variables on mean surface micro-hardness(SMH) at baseline, demineralization and after treatment being stored in artificial saliva are representing the static remineralized groups and ph cycling. There was no statistically difference between the groups at baseline p value (0.37) also and after being demineralized at p value(0.258). At static remineralization there was a statistically difference between the treated groups at $p<0.0001$. The group treated with laser and amelogenin peptide showed the highest SMH value with no statistically difference from the group which received the laser only, while the amelogenin peptide group showed the lowest statistically SMH value. However at pH cycling there was no statistically difference between the groups after being treated at $p = 0.67$.

Table (1) *Vickers surface hardness (Mean ±SD) values for enamel of all experimental groups at baseline, demineralized, and post-treatment at static remineralization*

Variables		Experimental group			ANOVA	
		Diode laser	Diode +amelogenin	Amelogenin peptide	F	P value
Treatment stage	Baseline	292.9± 14.08^{a}	299.8± 9.638^{a}	292.4±14.04^{a}	1.044	0.37ns
	Demineralized	217.9± 3.443^{c}	225.6± 7.038^{c}	219.3±17.19^{c}	1.426	0.258ns
	After static remineralization	258± 8.518^{Ab}	263.1±8.465^{Ab}	228.1±14.11^{Bb}	31.29	<0.0001*
ANOVA	F value	149.8	121.6	69.05		
	P value	<0.0001*	<0.0001*	<0.0001*		

*Superscripts with small letters indicates significant difference within the same column, Superscripts with capital letters for comparison within same row, *; significant ($p < 0.05$) ns; non-significant ($p > 0.05$)*

Table (2) *Vickers surface hardness (Mean ±SD) values for enamel of all experimental groups at baseline, demineralized, and post-treatment after being pH cycled*

Variables		Experimental group			ANOVA	
		Diode laser	Diode+amelogenin	Amelogenin peptide	F	P value
Treatment stage	Baseline	296.9±23.14^{a}	301.1±17.92^{a}	310.9±20.35^{a}	1.209	0.314ns
	Demineralized	218.3±18.01^{c}	222.9±14^{c}	223.2±16.96^{c}	0.776	0.759ns
	After ph cycling	258.2±44.91^{b}	260.1±27.4^{b}	248.2±16.58^{b}	0.404	0.67ns
ANOVA	F value	16.11	36.22	62.69		
	P value	<0.0001*	<0.0001*	<0.0001*		

Significant ($p < 0.05$) ns; non-significant ($p > 0.05$) superscripts with different letters indicates significant difference within the same column

Table (3) *Vickers hardness number (mean SD) for all tested groups remineralized in artificial saliva and after being pH cycled*

Treated Groups	Static remineralization	pH cycled	t value	P value
Diode laser	258± 8.518	258.2±44.91	0.01539	0.9ns
Diode + Amelogenin	263.1± 8.465	260.1±27.4	0.3383	0.74ns
Amelogenin peptide	228.1±14.11	248.2±16.58	2.915	0.0092ns

*significant ($p < 0.05$) *; significant ($p < 0.05$)*

DISCUSSION

Enamel is the hardest tissue in the human body-which consists of organic and inorganic components[20]. The organic matrix is composed of non collagenous protein which is 90% amelogenin [21]. In this study amelogenin peptide was used as a biomimetic material for evaluating its role in remineralization of incipient carious lesions.

Thirty human premolars were selected due to their availability because of orthodontic purposes. Flattening and polishing of the enamel surfaces was done to expose enamel surface with a higher porosity allowing enamel surface to be more susceptible to acid attacks and to remove natural inter sample variations on enamel surface to achieve standardization [10].

Assessment of enamel remineralization was done using micro-hardness testing. This test is applied for materials having fine microstructure, non homogenous and prone to cracking like enamel [22]. Therefore, it can reflect the mineral changes that have happened due to the surface treatment. It is considered a relatively a simple, non destructive and rapid method [23].

The results of the study showed that all surface pretreatment promoted remineralization of early enamel carious lesion. At static remineralization, the highest surface micro-hardness (SMH) values were found in by diode laser + Amelogenin peptide diode, followed by laser, and the lowest values were found in Amelogenin peptide.

The results showed that the highest SMH values found in the diode laser group + Amelogenin peptide, denoting a synergistic effect between physical method of surface alteration using lasers and chemical treatments using the amelogenin. However, the effect was not statistically significant in comparing to the laser group perse. This could attributed to that several proposed mechanisms of action induced by lasers. Among theses; laser irradiation leads to melting, fusion and resolidification of enamel crystals. Decreasing in water and Carbonate content, the rise in hydroxyl ion content and decomposition of enamel proteins are responsible against acid resistance provocations[13].

The results obtained in this study were in agreement with a previous study which found that application of laser with suitable parameters induced acid resistance of enamel [24], another study found that co_2 laser increased the enamel acid resistance and it also increased the surface micro-hardness [25].

The results of static remineralization were in contrary with a previous study that found higher remineralizing efficacy in the group treated with the remineralizing agent compared to the group which received laser treatment [14]. This contradiction could be attributed to different of the power used in both studies as in there study the power was set at 500 mw while at the present study was 200 mw. In addition difference in the remineralizing agents used in the previous study sodium fluoride while in the present study amelogenin peptide was used.

In contrary to a previous study that revealed sodium fluoride application and APF gel remineralizing agents showed higher significant surface micro-hardness compared to the diode laser treated group and the highest micro-hardness was recorded in the APF gel and diode laser [26]. This contradiction could be attributed to three reasons; First the wavelength used in this study was 970nm while in the present study was 810nm, second; difference in the remineralizing agents used in this study APF, third; sequence of application as in this study fluoride was applied first before laser while at the present study the laser was applied before the remineralizing agent.

The combination of diode laser and amelogenin peptide induced increasing in micro-hardness and remineralization of enamel carious lesion. This was in agreement with a study which revealed that application of laser followed by fluoride is effective in decreasing caries incidence [27],other study found that the surface micro-hardness significantly

increased when APF gel applied before laser [28]. This could be attributed to the thermal effect induced by laser primed the surface and increased the uptake of amelogenin peptide [25].This is in contrast with another study that showed treating enamel with laser and fluoride didn't give an additional effect for inhibiting demineralization of enamel [29].

The amelogenin group perse showed that there was a statistically significant increased in surface micro-hardness which was lower than the laser groups when exposed to static remineralization. However this effect diminished when exposed to ph cycling with no significant difference between the groups. The mechanism of action of amelogenin peptide depends on the concept of " Lego bricks" with the ability to spontaneously self assemble upon exposing to an external stimulus and environmental conditions of ph forming nano fibrous structure which are organized into 3D structures[8]. This 3D fibrillar scaffold enhances the attachment of calcium and phosphate ions form saliva causing increasing in the subsurface microhardness [30].

The increased values after being exposed to pH cycling as the self assembly peptide can be triggered by environmental fluctuations of PH and salt concentration forming a 3D scaffold which function as a nucleator for hydroxyapatite leading to tissue regeneration. At certain PH < 7.4 the peptide changes from a low viscosity isotropic liquid to an elastomeric nematic gel in which the anionic groups of the peptide side chains attract calcium ions, activate precipitation of new hydroxyapatite and promotes mineral gain insitu [31].

Remineralizing efficacy of self-assembly peptide was previously reported by previous studies [8,10,31] which found that self-assembly peptide induced remineralization of early enamel carious lesions. However, comparison between lasers and self-assembly peptides was not previously tackled in the literature.

PH cycling simulates the cycle which occurs naturally in the oral cavity as the ph decreases after meals leading to under saturation of the essential calcium and phosphate ions (ca_2 and po_4) in the plaque fluid while when the ph elevated near neutral the ions in saliva incorporate themselves into the depleted mineral layers of enamel as new apatite. The demineralized zones in the crystal lattice act as nucleation sites for new mineral deposition. The cycle depends on enamel solubility and ion gradients[32]. The aim of ph cycling is to measure the result of inhibition demineralization and the enhancement of remineralization as ph cycling simulates the high caries incidence in vivo [31].

The null hypothesis of the study was partially accepted as under static remineralization the use of laser was beneficial in remineralization compared to the use of amelogenin perse, meanwhile, under ph cycling, there was no difference between the tested groups.

CONCLUSION

Diode laser enhances the remineralization of incipient carious lesions compared to amelogenin peptides.

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RECOMMENDATIONS

1. Further in vivo studies are needed under clinical conditions.
2. The usage of laser should be used for remineralization as a mean of minimal invasive dentistry.

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CONFLICT OF INTEREST

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