

Effect of Er: Cr: YSGG Laser on the Remineralizing Potential of Self- Assembly Peptides in Incipient Carious Lesions (In Vitro Study)

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ABSTRACT

Background: Remineralization of incipient carious enamel lesions is an accepted biological model in restorative dentistry. Recently self-assembly peptides are gaining wide acceptance as being a biomimetic organic analogue simulating the natural amelogenesis procedure. In parallel, Er: Cr: YSGG lasers are thought to alter tooth substrate to be more recipient for subsequently applied remineralizing agent; however, a conjunction between lasers and self-assembly peptides was not tackled in literature. **Aim:** This study investigates the effect of Er:Cr:YSGG laser surface pre-treatment on the remineralizing potential of biomimetic self-assembling peptide (P11-4) on incipient carious lesion in terms of surface micro-hardness assessment. **Methodology:** Artificial enamel lesions were created on the buccal surface of 32 specimens, and were randomly allocated to four groups; G1: control- artificial saliva, G2: self-assembly peptide (Curodont Repair), G3: Er:Cr:YSGG laser surface treatment and G4: combination of Er:Cr:YSGG laser surface treatment followed by self-assembly peptide remineralizing agent application according to manufacturer's instruction. Surface microhardness (SMH) test was assessed at baseline, after demineralization and after treatment followed with pH cycling. Values were analyzed using ANOVA and Bonferroni's post-hoc test. **Results:** The highest statistically significant values of SMH were found in G4 followed by G2 and G3 while the lowest values were found in group G1. **Conclusion:** A synergistic effect was observed between laser as a surface pre-treatment and self-assembling peptide, showing superior enamel remineralization results with the highest surface micro-hardness measurements compared to the use of laser and self-assembling peptide alone.

Keywords: Self-assembly peptide, Er:Cr:YSGG laser, Remineralization, Enamel Regeneration.

INTRODUCTION

Caries is the most common disease burden upon communities especially the worldwide which is considered an economic developing ones.¹ It occurs due to an

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imbalance in the de/re-mineralization process. This dynamic process can be halted and reversed if detected and treated in the initial stages; however, if left without treatment it will lead to cavity formation.² Knowing this made a paradigm shift in the field of restorative dentistry. Halting and reversing the initial carious lesion is considered now a cornerstone in restorative dentistry.

Remineralization is the process by which calcium and phosphate ions are deposited into the crystal voids of demineralized enamel to form crystals.³ The bio mineralization process is the syngeneic effect of both organic and inorganic components enhancing the nucleation and growth of the inorganic counterpart to repair the damaged crystals.⁴ The challenge with remineralization is to provide the optimum concentration at the right time. If it is elevated at the wrong time, it can lead to unfavorable precipitation on the surface. Therefore limiting its efficacy, on the other hand, is slow and prolonged delivery might favor the subsurface mineralization.⁵

Among the organic analogues is the self-assembly peptide, which is a low viscosity monomer p11-4 that mimics enamel matrix protein. It forms a 3D bioactive scaffold in the subsurface of initial enamel carious lesion

by monomeric diffusion through the pores of demineralized enamel.⁶⁻⁸ It is capable of triggering the nucleation of de novo hydroxyapatite inducing mineral deposition in situ, self-assembling to fibers within the first 24 hours. The formation of non-prismatic hydroxyl apatite crystals results in an increase of surface micro hardness of enamel surface.⁹ In combination to mimicking the amelogenesis process; it is claimed that the inorganic nano-particles aggregation controls the crystal growth over the self-assembled organic scaffold.¹⁰ Hydroxyl apatite nucleate in the p11-4 self-assembly peptide scaffold, which diffuses into the body of the caries lesion to promote remineralization.^{6,11}

Despite the introduction of different products, literature was concerned with different surface pre-treatments claimed to enhance remineralization in conjunction with the products available.¹² Among those surface pre-treatments was the use of lasers which was claimed to cause crystalline changes in enamel.¹³ Laser absorbs water from the hydroxyapatite affecting its permeability and rendering it more acid resistant, arresting and preventing tooth caries.⁸ Ana et al.¹⁴ wrote a review article upon the laser effect on enamel for caries prevention. A synergistic effect was found

between the use of laser and fluoride application. The use of Er, Cr:YSGG laser induced an increase in the fluoride uptake and longer retention of the fluoride ions. It was also claimed that it induces a change in the polarization of enamel.¹⁴

Studies combining the effect of surface pre-treatment using lasers in conjunction with biomimetic Nano-organic analogues was not addressed in literature. Thus this study was carried out to examine the effect of laser surface pre-treatment on the remineralizing efficacy of the Nano-organic analogues using self-assembly peptides on incipient carious enamel lesions. The null hypothesis tested: there is no difference in the remineralization potential of incipient enamel lesions treated with self-assembly peptides, Er, Cr:YSGG laser and combination of their use in terms of micro hardness testing evaluation.

MATERIALS AND METHODS

This research was approved by ethical committee IRB# (00010118) at Misr International University. This in vitro investigation was carried out on 32 fresh sound extracted maxillary and mandibular human molars. The selected teeth were thoroughly cleaned, washed under running water; any calculus, blood and attached soft tissue debris were removed with ultra-sonic

scalers, then stored in distilled water at room temperature till use within maximum one month.¹⁵ Specimens were de-coronated by approximately 2 mm below the cemento-enamel junction using a microtome (Leica 1600 saw microtome, Wetzlar, Germany). The crowns were embedded in pink cylindrical self-cured acrylic resin molds (**Figure 1**). The buccal surfaces were wet polished flat with 800, 1200 and 2400 grit silicon carbide paper.

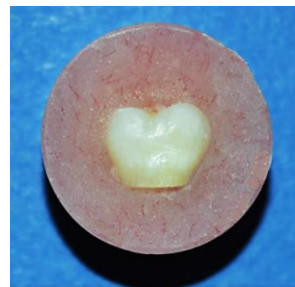


Figure (1): Tooth decoronated under CEJ by 2mm and embedded in cold cure acrylic resin.

Each specimen's buccal surface was covered with a square piece of adhesive tape (4*4 mm) and the entire remaining enamel surface was painted with a blue acid-resistant nail varnish (Jordana, Jordana cosmetic corp., dist. Los Angeles, USA). The adhesive tape was removed from the enamel surface once it had dried, leaving a square-shaped window of uniformly exposed enamel surface on each enamel specimen. (**Figure 2**)

In the exposed window of the enamel specimen's, the demineralizing solution (pH of 4.4 was achieved by adding 1 M potassium



Figure (2): Induction of white spot lesion.

hydroxide to a solution that included 2.2 mM calcium chloride, 2.2 mM sodium dihydrogen orthophosphate dehydrate, and 0.05 M acetic acid) was used to produce artificial white spot lesions.¹⁶ Each enamel specimen was individually immersed in 160 ml of the demineralizing solution, which was kept at room temperature for four days straight and then renewed every day, until a uniform white spot lesion was produced on the surface of the enamel window. Ten ml of the demineralizing solution was used per 1 mm² of enamel.¹⁶

Specimens numbered on the base of the acrylic block were randomly allocated into four equal group of eight specimens each. In the first control group (Group I), specimens did not receive a biomimetic material or any laser treatment. The second group (Group II) received self-assembly peptide Curodont repair treatment (Credentis AG, Windisch, Switzerland. LOT# CH180525-508). It is a synthetic self-assembling peptide (P11-4) made up of amino acids in a lyophilized state,

containing both CUROLOX technology for guided enamel regeneration and water for activation of the device) (**Figure3**).

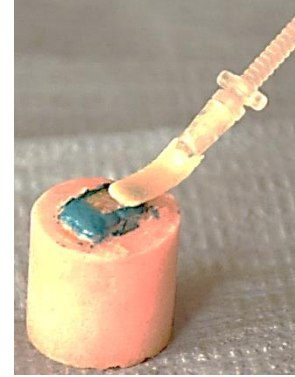


Figure (3): Application of self-assembling peptide Remineralizing agent.

In Group III, on the other hand, specimens were only treated with Er:Cr:YSGG laser for 10 seconds in non-contact mode, at a distance of two mm, with pulse duration of 60 microsecond at four Watt, 50 Hz energy (**Figure 4**).

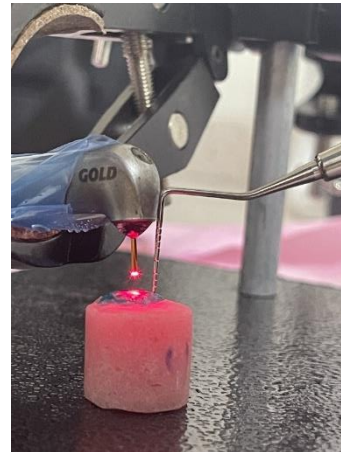


Figure (4): Enamel sample irradiated with Er:Cr:YSGG laser in a non- contact mode (2 mm distance).

Finally in Group IV, specimens were treated with both Er: Cr: YSGG laser for 10

seconds in non-contact mode, at a distance of two mm, with pulse duration of 60 microsecond at four-Watt energy (80% water 60% air), 50 Hz energy. This was followed by the self-assembly peptide (Curodont repair) application.

The 32 samples underwent individual pH cycles in separate containers for a total of 14 days, with a 4 day solution change in between the demineralization and remineralization phases. The proportions of demineralizing and remineralizing solutions per area of enamel were 6.25 mL/mm² and 3.12 mL/mm² respectively. The composition of the remineralizing solution (Chemically prepared solution containing 1.5mM CaCl₂, 0.9 mM NaH₂PO₄, 0.15 M KCl at pH 7).¹⁶ The specimens were individually submerged for 2 hours in 100 ml of a demineralizing solution, rinsed with deionized water, and then submerged for 22 hours each day in 50 ml of a remineralizing solution.^{17,18}

Surface Micro-hardness of the specimens was determined using Digital Display Vickers Micro-hardness Tester with a Vickers diamond indenter and a 20X objective lens. A load of 100g was applied to the surface of the specimens for 15 seconds. 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 specimen. The diagonals length of the indentations were measured by built in scaled microscope and Vickers values were converted into micro-hardness values. Micro-hardness was recorded at baseline, after demineralization and after remineralization. Micro-hardness was obtained using the following equation: $HV=1.854 P/d^2$. Percentage surface microhardness recovery %SMHR was calculated after remineralization as follows:¹⁹

$$SMH_R = \frac{VHN_F - VHN_D}{VHN_I - VHN_D} \times 100$$

Statistical Analysis: Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). All data showed normal (parametric) distribution. They were presented as mean and standard deviation (SD) values. Repeated measures ANOVA test was used to study the effect of surface treatment, De/Re-mineralization processes and their interactions on mean microhardness. One-way ANOVA test was used to compare between SMH_R values of the four surface treatments. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. The significance level was set at $P \leq 0.05$.

Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY:IBM Corp.

RESULTS

No significant difference was found between groups at baseline and after demineralization, while significant difference in surface micro-hardness was observed between baseline, demineralization and after treatment with pH cycling. In addition, significant difference between treatment groups was found with the highest surface micro-hardness value in Group IV, followed by Groups II and III, while the lowest values were in Group I as shown in **Table (1)**. Same was found for the % recovery shown in **Table (2)**.

DISCUSSION

Remineralization is considered a cornerstone in restorative dentistry. Several remineralizing agents are launched into the market; however, bio-mimetics are gaining wider acceptance. In the same track for enhancing remineralization and preserving the tooth structure, surface modifications were recommended by several authors. Among these were the use of dental lasers. However, the impact of laser was a controversial issue thus this study was conducted to elaborate the impact of using erbium chromium laser on the remineralization efficacy of the biomimetic self-assembly peptides.¹⁹⁻²⁵ Self-assembly

Table (1): The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between microhardness values with different interactions of variables.

De/Re-mineralization process	Group I (Control)		Group II (SAP)		Group III (laser only)		Group IV (laser +SAP)		P-value	Effect size (<i>Partial eta squared</i>)
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Base line	305.4 ^D	30.4	293.8 ^D	34.9	275.4 ^D	26.1	273.3 ^D	22.1	0.102	0.196
Demineralization	229.8 ^F	11.6	232 ^F	13	233.9 ^F	17	226.5 ^E	14.5	0.751	0.042
pH cycling	231.2 ^{CE}	11	247.9 ^{BE}	11.5	247.3 ^{BE}	8.3	262.1 ^{AD}	14.3	<0.00	0.51
P-value	<0.001*		<0.001*		0.008*		<0.001*			
Effect size (<i>Partial eta squared</i>)	0.726		0.473		0.298		0.715			

*: Significant at $P \leq 0.05$.

A,B,C Different superscripts in the same row indicate statistically significant differences between surface treatments.

D,E,F Different superscripts in the same column indicate statistically significant differences between De/Remineralization processes.

Table (2): The mean, standard deviation (SD) values and results of one-way ANOVA test for comparison between SMH_r (%) of the four surface treatments.

Group I (Control)		Group II (SAP)		Group III (Laser only)		Group IV (laser + SAP)		P-Value	Effect size (Eta squared)
Mean	SD	Mean	SD	Mean	SD	Mean	SD		
1.93 ^C	0.78	28.54 ^B	9.56	29.13 ^B	7.92	77.38 ^A	8.6	<0.001*	0.937

*: Significant at $P \leq 0.05$

Different superscripts indicate statistically significant differences between surface treatments.

peptides were evaluated in the present study since they were claimed by several authors to induce bio-mimetic remineralization simulating the natural amelogenesis process.²⁶⁻²⁸ Self-assembling peptide forms a 3D network template of a short hydrophilic peptide that assembles into fibers which attracts ions, triggering the nucleation of de novo hydroxyapatite crystals leading to guided regeneration of demineralized enamel.

Er:Cr:YSGG laser was designated in the present study since they have affinity for absorption in water and hydroxyapatite which are present abundantly in enamel.²⁹ In addition the parameters selected: pulse duration of 60 microseconds, average power, 4W energy (60% water 40% air), repetition 50 Hz, energy density 15.9 J/cm², tip in contact mode at a distance of 2 mm are claimed to induce sub-ablative surface modifications.³⁰

Extracted human molars were selected for this study for their wide surface area and

low surface convexity, which requires minimal amount of polishing to establish a flat area. Polishing of the specimens offers two benefits; first it eliminates prismless enamel layer that forms at the completion of amelogenesis and other natural variances in the surface. Second, it allows precise measurements for the surface micro-hardness testing.^{31,32}

In order to study the efficacy of self-assembly peptides, laser and their combinations on the remineralization of incipient carious enamel lesions pH cycling module was selected. Several pH cycling modules were presented in literature.^{17,33} However, the module selected in the present study was recommended by TenCate to represent low caries risk patients.^{34,35}

Vicker's micro-hardness test was used in the present study because Surface micro-hardness analysis (SMH) is an easy, quick and accurate way to assess surface resistance by giving indirect information regarding changes in enamel's mineral content and

hardness following demineralization and remineralization. SMH testing allows for repeated measurements of the same specimen over time and assesses the material's resistance to plastic deformation from a standard source.^{27,31,32,36}

The findings indicated that all three treatment regimens significantly promoted the remineralization of enamel lesions and increased enamel microhardness compared to artificial saliva. Thus, the null hypothesis has to be rejected.

The present study supports the forward-looking objective to induce biomimetic remineralization in incipient carious lesion, as the self-assembly peptide group induced significantly higher surface microhardness compared to the control group. This is in agreement with Suda, et al.,³⁷ Kamal, et al.,^{27,38} Sindhura, et al.,³⁹ and Alsamolly, et al.⁴⁰

The self-assembling peptide P11-4 is a short molecule with a rational design that in response to particular environmental stimuli, engages in hierarchical self-assembly into fibrillar scaffolds. Prior to the development of fibrils and edge-to-edge fibres, the peptide engages in one-dimensional self-assembly, resulting in the formation of micrometer-long nanotapes.³⁷ The nanotapes engages in a hierarchically predetermined process of self-

assembly in response to specific environmental factors, resulting in three-dimensional fibrillar scaffolds. Once constructed, these scaffolds act as excellent templates for hydroxyapatite nucleation, which promotes directed enamel regeneration. They mimic biological macromolecules known to regulate the deposition, shape, and growth of hydroxyapatite crystals that are found in extracellular matrices, such as those of the mammalian skeleton. This is attributed to the fact that the peptide is designed to be in monomeric liquid form when originally applied to the lesion's surface. This allows for diffusion thanks to its very low viscosity that ensures deep penetration into the subsurface lesion body micropores followed by a rapidly driven self-assembly promoting in-depth biomimetic remineralization. Triggering the process of self-assembly and gelation after its application is attributed to the low pH of the pH cycling model used in the present study. P₁₁₋₄ spontaneously switches and self-assembles to an elastomeric nematic 3D gels that show high affinity to tooth mineral, based on matching distances of Ca-binding sites on P₁₁₋₄ and Ca spacing in the crystal lattice of hydroxyapatite. This was backed by studies done by Kirkham et al. who found that after incubating P₁₁₋₄ in mineralizing

solutions for 7 days, needle-like electron-dense deposits appeared inside the scaffold itself. These deposits were thought to be crystalline hydroxyapatite.^{38,40}

According to Sindhura, et al.³⁹ one possible explanation for matrix-mediated mineralization provided by SAP_{P11-4} is via drawing calcium and phosphate ions from saliva and increasing the Ca: P ratio. Hydroxyapatite utilizes this as a nucleus to trigger tissue regeneration. A possible Ca²⁺ binding site is also provided by clusters of four Glu residues which are negatively charged while peptides assemble into fibres. Which contradicts Golland, et al.⁴¹ who established that the self-assembly peptide formed irregular hydroxyapatite crystals which did not promote remineralization in enamel significantly. This may be due to their use of bovine teeth in their study; bovine teeth differ from human teeth by having more carbonate and less fluoride, which induces faster formation of white spot lesion.³⁶

Results revealed that Er:Cr:YSGG laser pulse induced surface remineralization compared to artificial saliva. This is in agreement with Bharti et al,²⁹ Ana et al,¹⁴ and Qiao et al.⁴² who found that laser irradiation induce surface remineralization and decrease enamel solubility. Several theories were proposed: First heating of enamel from by

laser induced physical fusion of the enamel surface microstructure and sealing of the surface.^{42,43} The second theory was due to the chemical changes induced in enamel like the loss of organic matter, decomposition of protein, loss of water and carbonate contents.^{42,43} Another theory was the crystallographic changes in enamel like formation of resistant pyrophosphate crystals in place of hydroxyapatite crystals enhancing enamel's dissolution resistance.^{6,14}

By Contrast, Ulusoy, et al.²³ stated that laser application barely improved enamel resistance against demineralization compared to fluoride application. This contradiction may be due to the difference in the laser parameters used in their study compared to the present study 0.25 W– 4.42 J/cm², 0.50 W–8.84 J/cm², and 0.75 W–13.26 J/cm². They attributed their results to the formation of crystalline carbonate, forming a less stable and more soluble apatite. In addition, they relied on the measurement of the mass percentage of F which not only depends on the APF applied but also on the enamel's structural content and considered it to be the main factor responsible for the increase in enamel resistance. However, despite the appealing effect of combination of lasers and self-assembly peptides, clinical trials should be carried out to ascertain such effect.

CONCLUSION

Under the conditions of the present study, it can be concluded that: A synergistic effect was observed between laser as a surface pre-treatment and self-assembling peptide, showing superior enamel remineralization results with the highest surface micro-hardness measurements compared to the use of laser and self-assembling peptide persee.

FUNDING SOURCE

No funding source for this research.

CONFLICT OF INTEREST

No conflict of interest concerning this research.

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