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Evaluation of Effects of Er,Cr:YSGG Laser, Eggshell Solution, and Their Combination on the Remineralization of Erosive Lesion Caused by Lemonade

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Abstract

Introduction: Erosion is the loss of minerals by acids without the intervention of microorganisms. Complications caused by erosion make the use of therapeutic agents necessary. This study aims to survey the effects of the eggshell, the Er,Cr:YSGG laser, and their combination on the remineralization of erosive lesions caused by lemonade.

Methods: The microhardness of 40 impacted third molar teeth was measured in this laboratory study. Then, the samples were subjected to an erosion challenge using a regular lemonade drink. After measuring microhardness, the samples were exposed to remineralizing materials: Er, Cr:YSG laser, eggshell, laser Er,Cr:YSGG, eggshell, and fluoride (positive control), and artificial saliva (negative control). Finally, data were analyzed via Kolmogorov-Smirnov, mixed-model analysis of variance (ANOVA), and LSD post hoc tests.

Results: The microhardness of permanent third molar teeth, which was reduced after using lemonade, increased significantly after laser application compared to fluoride and artificial saliva. No significant difference was observed between the five experimental groups while there were statistically significant differences between the three stages of the study. Moreover, there was a significant interaction between the groups and stages (group * stage). The SMH of fluoride, artificial saliva, and laser+solution groups did not significantly change in the three stages of the study whilst the SMH of laser and solution groups significantly changed in the three stages. The SMH of the laser group had the most increasing trend (between erosive and treatment stages), followed by laser+solution and solution groups. However, the change in the laser+solution group was not statistically noticeable.

Conclusion: Based on the results of this study, the Er,Cr:YSGG laser alone can be effective in the remineralization of erosive lesions caused by lemonade.

Keywords: Er,Cr:YSGG laser, Eggshell, Erosion, Permanent teeth.

Introduction

Dental erosion is the irreversible destruction of dental hard tissue (enamel and dentin) through a chemical-mechanical process, unrelated to the activity of bacteria. One of the main causes of erosion is tooth exposure to acids.[1] The average prevalence of erosion in adults varies from 4 to 100% in different countries. Due to the variety of methods used in studies and differences in cultural and environmental factors, there is no consensus on the prevalence of erosion, leading to considerably different statistics in various countries. However, all studies agree that dental erosion is on the increase in all age groups due to the change in lifestyle.[2]

The etiology of dental erosion is multifactorial, with chemical, biological, and behavioral factors playing important roles. The erosive potential of acidic foods and drinks depends on chemical factors, such as pH, mineral content, and calcium chelation, and biological factors, such as saliva, acquired pellicle, tooth structure, and its position in relation to soft tissues and the tongue.[3] Erosion symptoms include cold and sweet sensitivity, pulp involvement, and tooth color change.[4]

Fluoride is one of the most effective re-mineralizing agents used to inhibit erosion. However, there are some concerns regarding the widespread use of fluoride. Chronic exposure to low levels of fluoride can cause digestive, genitourinary, and respiratory problems in healthy individuals. Furthermore, the prevalence of dental fluorosis has increased significantly in non-fluoridated areas and, to some extent, in optimally fluoridated areas.[5] Therefore, it is necessary to find a safe and efficient alternative to fluoride for the re-mineralization of eroded enamel lesions. Eggshell is a natural bio-ceramic with unique properties. Its chemical composition contains 91–

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94% calcium carbonate, 1% calcium phosphate, and other elements.[6,7] The results of a study showed that eggshells could be used as a re-mineralization agent in caries-like enamel lesions [5]. According to the results of a study, eggshell increases micro-hardness in third molars and finally improves the treatment of erosive lesions.[8] On the other hand, the wavelength produced by the Er,Cr:YSGG laser is well absorbed by water and the hydroxyl radical of hydroxyapatite, which indicates that the Er,Cr:YSGG laser has good potential to improve the acid resistance of the enamel.[9] In a study, the use of Er,Cr:YSGG laser in primary teeth was found to be effective in improving erosive lesions.[10]

This study was designed due to the complications of erosion and the necessity of its treatment, aiming to investigate the effect of eggshell and Er,Cr:YSGG laser on the remineralization of third molar erosive lesions caused by lemonade.

Materials and Methods

This experimental study was registered in the Ethics Committee of Shahed University, with the ethics code IR.SHAHED.REC.1400.133.

Production of Eggshell Powder

Chicken eggs were obtained from a local confectionary. Their contents were removed and the eggshells were cleaned in distilled water. The eggshells were then kept in a hot water bath at 100 °C for 10 minutes, followed by removing the membrane. These eggshells were then crushed using a sterile mortar and pestle. The crushed particles were then heated at 1200 °C in a muffle furnace and powdered into small particles. Next, 37 g of eggshell powder was dissolved in 740 ml of 4% acetic acid in a test tube. The clear fluid collected at the top was then transferred to a beaker, and the pH of the solution was tested using a pH meter, measuring 11.7.[11]

Laser Parameter

The irradiation conditions for the Er,Cr:YSGG laser were as follow: effective power of 0.5 W, medium power of 1 W, frequency of 20 Hz, cooling air/water spray (60% water, 40% air), wavelength of 2780 nm, pulse duration of 60 μs, and fluence of 8.84 J/cm^2.

Preparation of the Sample

A total of 40 (eight teeth in each group (laser, solution, laser-solution, fluoride, and artificial saliva) surgically extracted human impacted molars, which were free of enamel cracks, erosions, or hypoplastic lesions (confirmed by a stereomicroscope), were collected and stored in saline until testing. After cleaning, the teeth were mounted in a mold containing polyester, and the external enamel surface was polished using silicon carbide abrasive papers (2000, 1000, and 600) to assess surface microhardness (SMH).

Enamel SMH Tests

After polishing, the baseline SMH of the teeth was measured using a Vickers microhardness tester (Type Zwick Roll Corporation, England) with a 50 g load for 10 seconds.[11]

Erosive Challenge

Each enamel sample was rinsed with distilled water for 10 seconds and immediately immersed into 6 mL of fresh lemonade (pH: 3.062) (Zamzam Company, Iran) in a plastic container for 4 intervals of 2 minutes (a total of 8 minutes) [12], and then it was rinsed again with distilled water and stored in a fresh artificial saliva solution (1.5 mmol/L of calcium, 0.9 mmol/L of phosphorus, and 150 mmol/L of potassium chloride- Research Center, Faculty of Dentistry, Tehran University of Medical Sciences). The erosive challenges were applied at 0, 12, 24, 36, 48, and 60 hours. The pH of the soft drink was measured using a digital pH meter and kept constant at room temperature for at least 15 minutes (pH = 3.062). If the microhardness decreased significantly, it meant that the erosive challenge was successful.

Enamel SMH Tests

The SMH of each enamel specimen was evaluated after erosive challenge with the same device and under the same load (SMH2).

Surface Treatments

For group 1 (control group), specimens were stored in artificial saliva. For group 2 (F), acidulated phosphate fluoride (1.23%, pH: 3.6– 3.9) (Iran's Nik Darman APF Fluoride Gel) was applied with a micro brush for 10 minutes. In group 3 (L), the samples were irradiated using a pulsed Er, Cr:YSGG laser (Biolase; America) with a Gold handpiece, a checkerboard movement, and a laser tip diameter of 0.2 cm, at a wavelength of 2,780 nm, with the following parameters: effective power of 0.5 W, frequency of 20 Hz, cooling air/water spray (60% water, 40% air), wavelength of 2780 nm, pulse duration of 60 μs, fluence of 8.84 J/cm^2, and pulse energy of 25 mJ in the hard mode for 15 seconds. In group 4 (EGS + L), the samples were immersed in the eggshell solution for 10 minutes, followed by laser irradiation for 10 seconds. In group 5 (EGS), the samples were immersed in the eggshell solution for 10 minutes.

Enamel SMH Tests

The SMH of each enamel specimen was evaluated after surface treatment with the same device and under the same load (SMH3).

Statistical Analysis

After collecting the desired data, the normal distribution was evaluated via the Kolmogorov- Smirnov test.

Thereafter, the data among five groups in three different stages (baseline, erosive challenge, and treatment) of the study were compared using the mixed-model analysis of variance (ANOVA), followed by the LSD post hoc test for pairwise comparisons (α = 0.05).

Results

Descriptive statistics are described in Figure 1. The mixed-model ANOVA revealed no significant differences between the five experimental groups ($P = 0.79$), while there were statistically significant differences between the three stages (baseline, erosive challenge, and treatment) of the study ($P = 0.000$). Moreover, the interaction between groups and stages (group*stage) was significant ($P = 0.000$).

The mean ± SD of the five groups in the three stages is depicted in Figure 1. According to this figure, in conjunction with the statistical analysis (the estimate of fixed effects), the SMH of fluoride, artificial saliva, and laser + solution groups did not significantly change in the three stages of the study ($P = 0.45$, 0.33, and 0.07, respectively). However, the SMH of the laser and solution groups changed significantly in the three stages ($P = 0.003$ and 0.000, respectively).

As shown in Figure 1, the SMH of the laser group has the most increasing trend (between erosive and treatment stages), followed by the laser + solution and solution groups. However, the change in the laser + solution group was not statistically noticeable.

Moreover, none of the pairwise *P*-values of the groups were significant, as demonstrated in Table 1.

Discussion

Dental erosion is a matter of concern due to its increasing prevalence and severity in recent years.[13] Erosion processes lead to surface demineralization and then complete destruction of the surface of the enamel. Considering the side effects of erosion, it seems necessary to search for a substance that could accelerate the remineralization of the enamel. In this study, the effect of Er,Cr:YSGG laser, the eggshell solution, and the combination of the two on impacted third molar permanent tooth enamel was investigated in situ after exposure to lemonade.

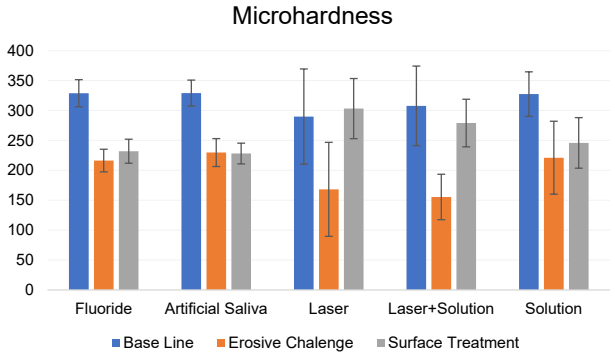

Figure 1. The mean ± SD of Five Experimental Groups in Three Stages of the Study

The results of this study show that the micro-hardness of teeth decreases after exposure to lemonade.

Lemonade is completely acidic, with a pH of 3.062, a pH lower than the critical limit of enamel (5.5); in other words, lemonade dissolves hydroxyapatite crystals in the enamel, which ultimately reduces the micro-hardness of the tooth.

Some studies have shown that the use of any carbonated drinks, such as beer or soft drinks with an acidic pH, causes erosion and decreases tooth micro-hardness.[5,8,10]

Based on the results of this study, the Er,Cr:YSGG laser significantly increased the microhardness of permanent molar teeth compared to fluoride and artificial saliva.

Laser radiation reduces the permeability and solubility of enamel through crystallographic changes. Carbonates and organic materials (water and protein) between the enamel prisms decrease with the melting and fusion of enamel crystals.

The overall resistance of enamel against acids increases with the recrystallization of enamel crystals, an increase in the size of crystallites, and the formation of tricalcium phosphate and tetracalcium phosphate. Crystallographic changes in enamel occur due to the heat generated by the laser. The destruction of enamel matrix materials causes the blocking of the prismatic space, which reduces ion diffusion and delays demineralization.[14-16]

There are two ideas about how the laser works. The first theory is that the buildup of calcium, phosphate, and fluoride ions increases in the porosity and a fissure is created by the laser. Based on the second hypothesis, heat generated by the laser causes fluoride to enter hydroxyapatite and fluorapatite crystals. In the first hypothesis, the bond between the ions and the tooth structure is weak. In the second theory, however, there is a strong link. Furthermore, the rate of mineralization increases as a result of released water, organic compounds, and carbonate ions from the hydroxyapatite structure due to heat generation in the area. In aqueous and hydroxyapatite tissues, the Er,Cr:YSGG laser (wavelength 2780 nm) has the highest absorption rate and hydroxyapatite crystals in the enamel, thereby increasing the possibility of remineralization.

Table 1. *P* Values Related to the Pairwise Comparison of Five Experimental Groups

	Fluoride	Artificial Saliva	Laser	Laser + Solution	Solution
Fluoride	-	0.83	0.72	0.45	0.71
Artificial Saliva	-	-	0.57	0.33	0.87
Laser	-	-	-	0.68	0.47
Laser + Solution	-	-	-	-	0.26
Solution	-	-	-	-	-

The results of this study showed that the eggshell solution increased the microhardness of demineralized teeth, but there was no significant difference between laser and fluoride. Eggshell is a natural bioceramic and has unique properties [7]. The chemical composition of eggshells contains 91%-94% calcium carbonate, 1% calcium phosphate, and other elements.[17] Eggshell powder is an abundant biological source of calcium (401 mg), containing high levels of strontium (372 ug) and low levels of zinc, copper, magnesium, nitrogen, and copper.[18] Thus, it has the potential for the remineralization of erosive lesions.

In our study, the laser in combination with the eggshell solution had a lower effect than the laser group alone, which can be due to the priority of using the eggshell solution before the laser.

Using the eggshell before the laser may create a mechanical barrier that reduces the temperature and energy of the enamel surface and prevents any change in it.[19]

The results of various studies show that the simultaneous use of fluoride along with the Er,Cr:YSGG laser can increase the therapeutic effect of the laser[20]; however, there are also studies claiming that the use of these materials before or after the laser has no effect.[21] Moreover, some other studies report that it is better to use these materials after using the laser as the use of these materials before the laser may create a mechanical barrier that reduces the temperature and energy of the enamel surface and prevents any change in the enamel.[19]

In this study, the effects of the laser and eggshell and the combination of the two were compared on the remineralization of permanent tooth enamel for the first time. Therefore, no similar study has been conducted to compare the results of this study.

Yilmaz et al showed that the highest increase in remineralization was achieved by the Er,Cr:YSGG laser group at a power of 0.5 and a frequency of 20 Hz, which is consistent with the results of our study, with the difference that cow's teeth were used in the study of Yilmaz et al, and their pH-cycling cycle was different from that of the present study.[10]

In contrast to our study, AlShamrani et al showed that the Er,Cr:YSGG laser alone (a power of 0.5 and a frequency of 20 Hz) was not effective in controlling erosion. They also stated that laser radiation before using APF GEL could be effective in treating erosive lesions.[19] This could be due to the difference in the pH-cycling method.

Sadabadi et al showed that the Er,Cr:YSGG laser alone (power of 0.75 W, frequency of 20 Hz, 60% water, 40% air, and pulse duration of 140 μs) was not effective in erosion remineralization compared to Novamin and laser-Novamin; these results are not consistent with our study.[22] This difference can be due to the difference in the demineralizing material and its application time, as well as the laser setting in the two studies.

Mony et al reported that the eggshell solution could remineralize enamel lesions.[11] This is similar to our study, with the difference that the laser in combination with the eggshell solution as a therapeutic agent was used here, and the teeth were exposed to the therapeutic agent for a shorter period of time. The results of a study by Haghgoo et al showed that different concentrations of the eggshell solution were equally able to re-mineralize decay-like lesions.[5] This study is similar to our study research, with the difference that our study measured remineralization using the laser in combination with one concentration of the eggshell solution as a therapeutic agent, and the therapeutic agents were applied to the teeth for a shorter period of time.

Moslemi et al showed that laser in combination with fluoride reduced enamel demineralization more than laser or fluoride alone, regardless of the sequence. Moslemi et al used a lower power and a shorter pulse time; they utilized the reverse of the procedure used in this study, and the water-air ratio was 0/11 in the laser mode.[20]

The effects of Er,Cr:YSGG laser, the eggshell solution, and the combination of the two on the remineralization of dental erosion caused by lemonade were evaluated in this study. It is recommended to evaluate the effect of other re-mineralizing materials, such as ACP-CPP, in combination with a laser in view of re-mineralization. It is also recommended to study the order of application of materials and lasers.

Conclusion

Based on the results of this study Er,Cr:YSGG laser alone can be effective in the remineralization of erosive lesions caused by lemonade.

Authors' Contribution

Conceptualization: Roza Haghgoo, Fatemeh Dehghani.
Data curation: Fatemeh Dehghani, Mohammad Asnaashari.
Formal analysis: Fatemeh Dehghani, Roza Haghgoo.
Investigation: Fatemeh Dehghani, Roza Haghgoo.
Methodology: Roza Haghgoo, Fatemeh Dehghani.
Project administration: Fatemeh Dehghani, Hossein Dehghan.
Resources: Hossein Dehghan.
Software: Hossein Dehghan.
Supervision: Roza Haghgoo, Fatemeh Dehghani.
Validation: Roza Haghgoo.
Visualization: Hossein Dehghan.
Writing–original draft: Fatemeh Dehghani.
Writing–review & editing: Roza Haghgoo.

Competing Interests

There is not any conflict of interest.

Ethical Approval

This research was approved by the research ethics committee of Shahed University of Tehran (IR.SHAHED.REC.1400.133) and there were not any ethical considerations.

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