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Effects of Er,Cr:YSGG Laser Irradiation, Application of Eggshell Solution, and a Combination of Both on Enamel Resistance against Lemonade-Induced Erosion

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A B S T R A C T

Objectives: This study assessed the effects of erbium, chromium: yttrium, scandium, gallium, and garnet (Er,Cr:YSGG) laser irradiation and eggshell solution on enamel resistance to lemonade-induced erosion.

Materials and Methods: This in vitro study was conducted on 40 surgically extracted mandibular third molars. After initial microhardness measurement, the specimens were assigned to five groups (n=8), including three experimental and two control groups. The experimental group specimens received (I) Er,Cr:YSGG laser irradiation, (II) eggshell solution, and (III) Er,Cr:YSGG laser plus eggshell solution. The positive and negative controls were fluoride and artificial saliva, respectively. Microhardness was reassessed after treatment, erosion, and pH cycling. Data were analyzed by repeated measures ANOVA, Tukey's test, and paired t-test (alpha=0.05).

Results: After fluoride application, microhardness slightly increased compared to baseline (0.92%); whereas a decrease in microhardness was observed in other groups (-6.71% for laser, -1.78% for laser + eggshell solution, -6.01% for eggshell solution and -10.54% for artificial saliva). Changes in microhardness at different steps were insignificant in the fluoride (P=0.09) and eggshell (P=0.09) groups. A statistically significant descending trend was observed in microhardness of laser (P=0.003), and laser + eggshell (P=0.02) groups but the microhardness change at different steps was not significant in the artificial saliva group (P=0.34).

Conclusion: An increase in microhardness was observed in the fluoride group after remineralization, and its microhardness reduction was not significant after demineralization. Fluoride was more effective than artificial saliva, Er,Cr:YSGG laser, eggshell solution, and eggshell solution plus Er,Cr:YSGG laser in enhancing enamel resistance to lemonade-induced erosion.

Keywords: Egg Shell; Lasers; Tooth Erosion

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INTRODUCTION

Dental erosion is the gradual process of irreversible degradation of enamel and dentin that can be accredited to a chemical procedure with no contribution of bacteria [1]. Dental erosion is a multifactorial process, caused by a combination of biological, chemical, and behavioral factors. Erosion may occur with the collective contribution of acidic foods and drinks such as lemonade, which is a common dietary risk, contingent upon their pH, mineral level, and calcium chelation, alongside biological factors, e.g. saliva, tooth structure, and tooth position in relation to soft tissues and the tongue [2]. Dentin hypersensitivity, pulpal involvement, sensitivity to cold and sweets, and tooth discoloration are the consequences of dental erosion [3]. The adversative aftermaths of dental erosion necessitate its prevention and timely diagnosis. Protective approaches comprise stimulating saliva flow, dietary control, fluoride therapy, laser therapy, and applying calcium phosphate compounds [4].

Fluoride therapy is a highly effective protective intervention for dental erosion. Nevertheless, extreme exposure to fluoride raises some concerns. Chronic exposure to low doses of fluoride can result in respiratory, gastrointestinal, and renal complications. Contrarily, fluorosis is less prevalent in non-fluoridated geographical areas and satisfactory in areas where fluoridation is performed [5]. Thus, a safe alternative solution should be found to prevent dental erosion.

Eggshell as a calcium-rich source can be a good substitute for fluoride. Eggshell possesses calcium carbonate (94%), calcium phosphate (1%), magnesium phosphate (1%), and organic matter (4%). Eggshell powder was first utilized as a curative substance in 1992 [6]. In an earlier investigation, eggshell was utilized because of its optimal biocompatibility as an organic source of phosphate and calcium to remineralize enamel carious lesions [5]. Additionally, eggshell could increase the microhardness of third molars, eventually resolving their erosive defects [7].

Water, hydroxyapatite, and hydroxyl radicals efficiently absorb the wavelength generated by erbium, chromium: yttrium, scandium, gallium, and garnet (Er,Cr:YSGG) laser. De Oliveira et al. [8] reported good potential of Er,Cr:YSGG laser to enhance enamel resistance to acid attacks.

Previous studies investigating the efficacy of eggshell and laser for restoring carious lesions reported promising results [5,6]. However, the effect of a combination of eggshell and laser application on erosive lesions has not been previously investigated.

Given the importance of preventing erosive enamel lesions and the promising results of laser therapy and eggshell solution application, the current study focused on assessing the impacts of Er,Cr:YSGG laser irradiation, eggshell solution application, and their combined use on enamel resistance against lemonade-induced erosion.

MATERIALS AND METHODS

This in vitro study was performed on sound surgically extracted mandibular third molars. It received approval from the ethics committee of Shahed University (IR.SHAHED.REC.1400.133). The teeth had been extracted for purposes not related to this study, and belonged to patients between 18-21 years. All patients consented to the use of their extracted teeth for possible research purposes. The sample size was calculated to be 8 in each group (a total of 40) using the repeated measures ANOVA feature of G Power software (five groups, three measurements) considering alpha=0.05, beta=0.2, and effect size=0.5.

Preparation of specimens:

The collected 40 extracted third molars underwent clinical examination under a stereomicroscope to ensure absence of caries. They were then blindly and randomly assigned to five groups (n=8). The teeth were stored in saline at ambient temperature until the experiments. The teeth were debrided and disinfected by immersion in chloramine-T solution for 3 days. After mounting the teeth in acrylic resin, the enamel surface was uniformly polished with 600-, 1000-, and 2000-grit silicon carbide abrasive papers (Matador Wasser Quick, Germany) to remove the outer fluoride-rich enamel layer, which could affect the baseline microhardness. An area measuring 4×4mm remained untouched in the middle, and the surrounding area was covered with an acid-resistant varnish. Initial microhardness of the specimens was then measured by a Vickers hardness tester (Zwick, UK) [9]. For this purpose, indentations were made at three random points on the surface by application of 50g force for 10 seconds perpendicular to the enamel surface. All the procedures were performed by one operator.

Preparation of eggshell powder:

The eggshells were obtained as explained by Mony et al [5]. They were rinsed with distilled water, cleaned, and then boiled at 100C for 10 minutes for membrane removal. Next, the eggshells were powdered into small particles with a coffee grinder, and the eggshell powder (70g) was heated in a furnace at 1100°C for 50 minutes, producing 37g of powder, which was dissolved in 4% acetic acid (740mL). After a few minutes of settling the powder, the clear liquid over the test tube was collected in a tube to measure its pH which was 11.9 [5].

Laser parameters:

The Er,Cr:YSGG laser parameters were as follows: 2,780nm wavelength, 0.5W mean power, 20Hz frequency, 60µsec pulse duration, 8.84J/cm^2 fluence, and 25mJ pulse energy in the hard mode for 15 seconds with a laser tip diameter of 0.2cm and 60% water/40% air cooling. Then, the specimens were assigned to the following five groups (n=8):

-*Group 1 (positive control):* The specimens were immersed in 1.23% acidulated phosphate fluoride (ph=3.6–3.9) for 10 minutes.

-*Group 2 (negative control):* The specimens were immersed in artificial saliva containing calcium (1.5mM/L), phosphorus (0.9mM/L), and potassium chloride (150mM/L) (Research Center, Faculty of Dentistry, Tehran University of Medical Sciences) for 10 minutes.

-*Group 3:* The specimens were immersed in a 5% eggshell solution for 10 minutes [7].

-*Group 4:* The specimens were exposed to Er,Cr:YSGG laser (Biolase; USA) using a gold handpiece with checkerboard movement [10].

-*Group 5:* The specimens were immersed in a 5% eggshell solution for 10 minutes and irradiated with Er,Cr:YSGG laser as explained for group 4.

The aforementioned interventions were followed by remeasuring all specimens' microhardness. Indentations were spaced adequately (>100µm apart) to avoid overlapping of stress areas. Then, the specimens were immersed in a lemonade solution (Zamzam Company, Tehran, Iran) which included sugar, citric acid, sodium benzoate, natural lemon taste, quinoline yellow, and ester gum (pH=3.06) for 2 minutes at 0, 12, 24, 36, 48, and 60 hours after pH cycling [7]. Each specimen was rinsed with distilled water and stored in artificial saliva for the respective period. Finally, their microhardness was measured again after completion of pH cycling. Microhardness was measured at the same points measured in previous steps (marked areas) to avoid the confounding effects. All steps were performed by one operator who was blinded to reduce measurement bias.

Data normality was assessed using the Shapiro-Wilk test, which showed that the microhardness values in all groups had a normal distribution (P>0.05 for all groups). Since the microhardness values had to be compared among the five groups in three periods, repeated measures ANOVA was applied for the comparisons followed by pairwise comparisons with the Tukey's post-hoc test. Paired t-test was also used for pairwise comparisons between the stages (time points) within each group. P≤0.05 was considered statistically significant.

RESULTS

This study evaluated the enamel resistance of 40 teeth to erosive lesions induced by lemonade following exposure to eggshell solution, fluoride, laser therapy, and combined eggshell solution and laser therapy in five groups (n=8). The mean enamel microhardness following exposure to the remineralizing agents and after pH cycling is reported in Table 1.

After the application of fluoride, the microhardness experienced a slight increase compared to baseline (percentage of change=0.92%); whereas a decrease was observed in microhardness of other groups (percentage of change=-6.71% for laser, -1.78% for laser + eggshell solution, -6.01% for eggshell solution, and -10.54% for artificial saliva).

At baseline after preparing the specimens, there was no statistically significant difference among the groups in microhardness (P>0.05),

Table 1. Mean enamel microhardness following exposure to remineralizing agents and after pH cycling

Assessment time	Group	Mean	SD	SE	Min	Max
Baseline: After preparing the specimens	Fluoride	326.00	48.49	17.14	245	404
	Laser	313.75	62.89	22.24	246	411
	Laser + eggshell solution	281.13	32.20	11.39	227	319
	Eggshell solution	283.00	46.36	16.39	233	354
	Artificial saliva	351.13	34.50	12.20	301	416
After remineralization	Fluoride	329.38	57.10	20.19	230	396
	Laser	292.63	47.70	16.86	201	349
	Laser + eggshell solution	276.88	31.23	11.04	232	324
	Eggshell solution	266.00	39.84	14.09	217	338
	Artificial saliva	314.63	37.28	13.18	252	369
After demineralization	Fluoride	292.00	42.98	15.19	236	343
	Laser	239.63	30.15	10.66	190	276
	Laser + eggshell solution	222.13	46.81	16.55	183	261
	Eggshell solution	227.50	41.57	14.70	161	267
	Artificial saliva	318.00	71.24	25.19	184	403
	Total	259.85	60.04	9.49	146	403

SD: Standard deviation; SE: Standard error; Min: Minimum; Max: Maximum.

except that the artificial saliva group had a significantly higher microhardness than laser + eggshell solution (P=0.04), and eggshell solution (P=0.04) groups. After remineralization, a statistically significant difference was found only between the fluoride group and the eggshell solution group (P=0.05). After demineralization, enamel microhardness of the artificial saliva group was significantly higher than that of eggshell solution (P=0.006), and laser + eggshell solution groups (P=0.003). Moreover, in this stage, fluoride group had a significantly higher microhardness than laser + eggshell solution group (P=0.05). The fluoride group also showed significantly higher microhardness compared to the laser + eggshell solution group (P=0.05), and insignificantly higher than the eggshell solution (P=0.08) group (Table 2).

Repeated measures ANOVA was used to assess the trend of microhardness change over time across the study groups (Fig. 1). Based on this analysis, following fluoride application, enamel microhardness exhibited a slight increase; whereas a decrease was observed in other groups. After demineralization, microhardness decreased in all groups; however, in the artificial saliva group, a slight increase was observed compared to the remineralization phase. Changes at different experimental steps in the fluoride (P=0.09) and eggshell (P=0.09) groups were not significant. A statistically significant decreasing trend was observed in the laser (P=0.003) and laser + eggshell solution (P=0.02) groups. No significant change in microhardness was recorded in the artificial saliva group (P=0.34; Fig. 1).

Pairwise comparisons by paired t-test are presented in Table 3. As shown, in the fluoride group, there was no significant difference in

Table 2. Pairwise comparisons of the study groups regarding enamel microhardness

Group 1	Group 2	Microhardness after specimen preparation			Microhardness after remineralization			Microhardness after demineralization		
		Mean Diff. *	SE	P value	Mean Diff.*	SE	P value	Mean Diff. *	SE	P value
Fluoride	Laser	12.25	23.11	0.98	36.75	21.78	0.45	52.38	24.24	0.22
	Laser + eggshell solution	44.88	23.11	0.32	52.50	21.78	0.14	69.88	24.24	0.05
	Eggshell solution	43.00	23.11	0.36	63.38	21.78	0.05	64.50	24.24	0.08
	Artificial saliva	-25.13	23.11	0.81	14.75	21.78	0.96	-26.00	24.24	0.82
Laser	Laser + eggshell solution	32.63	23.11	0.62	15.75	21.78	0.95	17.50	24.24	0.95
	Eggshell solution	30.75	23.11	0.67	26.63	21.78	0.74	12.13	24.24	0.99
	Artificial saliva	-37.38	23.11	0.49	-22.00	21.78	0.85	-78.38	24.24	0.02
Laser + eggshell solution	Eggshell solution	-1.88	23.11	1.00	10.88	21.78	0.99	-5.38	24.24	0.10
	Artificial saliva	-70.00	23.11	0.04	-37.75	21.78	0.43	-95.88	24.24	0.003
Eggshell solution	Artificial saliva	-68.13	23.11	0.04	-48.63	21.78	0.19	-90.50	24.24	0.006

* Mean difference between the compared groups; SE: Standard error.

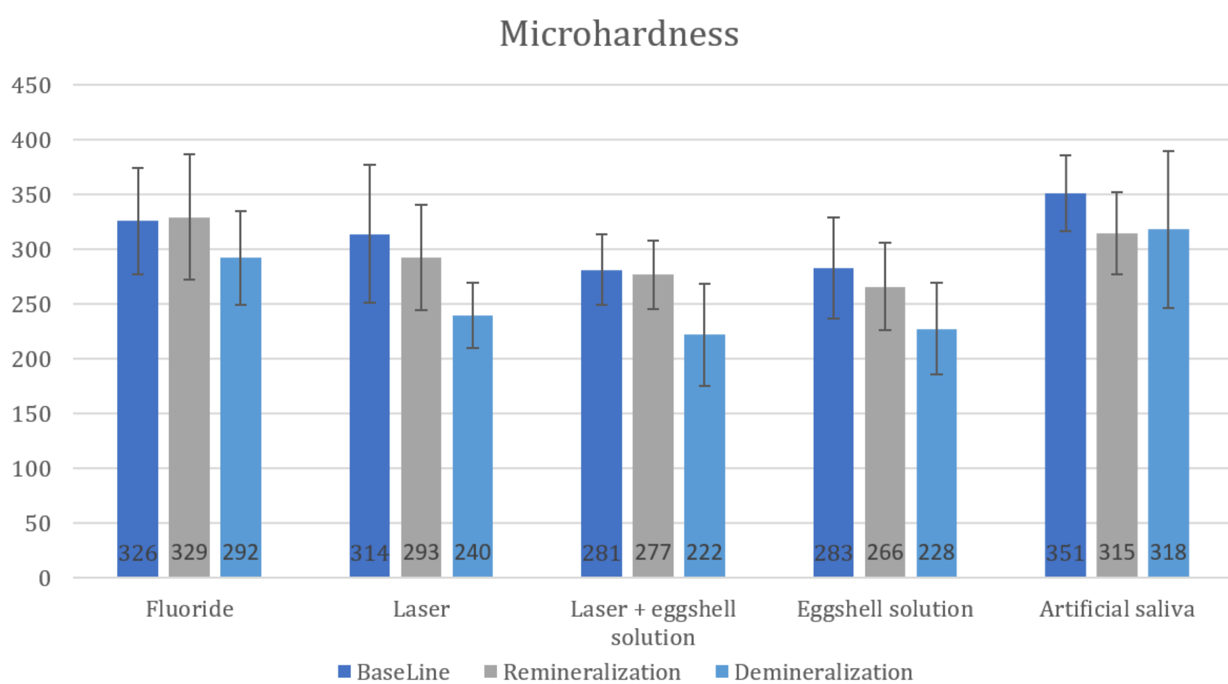

Fig. 1. Mean microhardness values over time in the study groups.

Table 3. Pairwise comparisons of microhardness values at different stages within each study

Group	Stage	Mean	SD	P value*
Fluoride	BL–RM	-3.38	41.23	0.82
	RM–DM	37.38	53.37	0.08
	BL–DM	34.00	50.39	0.10
Laser	BL–RM	21.13	46.72	0.24
	RM–DM	53.00	33.09	0.003
	BL–DM	74.13	65.40	0.02
Laser + eggshell solution	BL–RM	4.25	49.75	0.82
	RM–DM	54.75	66.78	0.05
	BL–DM	59.00	51.14	0.01
Eggshell solution	BL–RM	17.00	51.28	0.38
	RM–DM	38.50	73.17	0.18
	BL–DM	55.50	75.16	0.08
Artificial saliva	BL–RM	36.50	54.11	0.10
	RM–DM	-3.38	98.36	0.93
	BL–DM	33.13	62.85	0.18

*Paired t-test; SD: Standard deviation; BL: Baseline; DM: Demineralization; RM: Remineralization.

microhardness when comparing the microhardness after demineralization and after remineralization stages (P=0.09). In the laser group, there was a statistically significant remineralization stages (P=0.09). In the laser group, there was a statistically significant difference in microhardness when comparing the microhardness values after demineralization and after remineralization stages (P=0.003), and when comparing microhardness values after demineralization and baseline (P=0.02). In the laser + eggshell solution group, significant differences were found between microhardness values after demineralization and after remineralization (P=0.01), and also between microhardness values after demineralization and baseline (P=0.01). In the eggshell solution group, an insignificant difference was found between microhardness values after demineralization and baseline (P=0.08). In the artificial saliva group, remineralization did not have a significant effect on enamel microhardness (P>0.05).

DISCUSSION

Due to the adversative aftermaths of enamel erosion, the current study assessed the impact of Er,Cr:YSGG laser irradiation and application of eggshell solution on third molars' enamel resistance to lemonade-induced erosion. Previously, an investigation on the therapeutic impacts of laser irradiation and applying remineralizing agents on enamel erosive lesions discovered the promising potential of laser therapy in reversing erosive enamel lesions [11]. Nevertheless, the authors' literature review disclosed no study comparing the inhibition of enamel erosive lesions by laser therapy and applying eggshell solution as a remineralizing agent. The current study apparently pioneers in addressing this subject.

Eggshell is a natural bioceramic with unique properties. The chemical composition of eggshell includes 91%-94% calcium carbonate, 1% calcium phosphate, and other elements. Eggshell powder is an abundant biological source of calcium (401mg), containing high levels of strontium (372ug) and low levels of zinc, copper, magnesium, nitrogen, and copper. Thus, it has the potential for remineralization of erosive lesions [12].

Based on the findings of the present study, only the fluoride group exhibited an increase in microhardness. Although this increase was not statistically significant, it indicates the potential effectiveness of fluoride for this purpose. As shown in the literature, applying fluoride can effectively hinder dental erosion due to its increased enamel resistance to acid attacks [13], which confirms our observations. The present findings further demonstrated improvement of enamel resistance to acid attack through laser irradiation, which can physically fuse enamel components. Furthermore, enamel crystals better reconnect and recrystallize due to reduced permeability and dissolution of enamel caused by melting. The decrease in enamel dissolution is attributable to laser-generated heat that induces alterations in enamel crystallography [14]. The carbonates and organic components (water and proteins) are reduced due to crystallographic changes in the enamel structure resulting from laser-generated heat. Moreover, the crystal size is enlarged, and tri-calcium phosphate, tetra-calcium phosphate, and hydroxyl ions are formed. Such alterations play a role in generally improved enamel resistance to acid attacks [15,17].

The prismatic and inter-prismatic spaces are closed by removing organic ingredients from the enamel matrix, preventing diffusion between the prisms and resulting in reduced release of ions and delayed demineralization. Such an event happens when the enamel temperature rises to 400°C. In the present study, the effects of this temperature were investigated using a fluence of 8.84J/cm^2. It is worthy of note that enamel removal at an approximate threshold of 10-14J/cm^2 negates the peril of enamel destruction at this fluence [18].

Unlike the fluoride group, all other groups demonstrated a decrease in microhardness. The greatest reduction was observed in the artificial saliva group, followed by the laser and eggshell solution groups. The least reduction was noted in the group treated with laser + eggshell solution. Therefore, it is an effective substance after fluoride to confer acid resistance to enamel.

As mentioned above, eggshell is a rich source of calcium. It has also been reported to improve enamel resistance to acid attacks [10]. The combination of eggshell solution with Er,Cr:YSGG laser appears to be effective in this regard; however, no previous studies were found in the literature to compare our results with.

The present findings indicate that the combined eggshell solution plus laser was significantly different from the fluoride and laser irradiation groups. The combination of eggshell solution and Er,Cr:YSGG laser was apparently efficacious in this respect; yet, this result cannot be compared as no similar previous study was found in the literature.

Unlike our observations, one single round of Er,Cr:YSGG laser with 0.5W power and 30Hz frequency could properly enhance enamel resistance in a previous study by AlShamrani et al [19]. The discrepant findings might result from differences in the erosive challenge procedure. It is also noteworthy that AlShamrani et al. [19] utilized bovine teeth, and different tooth types (human vs. bovine) might justify the discrepant findings. Dionysopoulos et al. [20] observed positive effects of Er,Cr:YSGG laser irradiation with 0.25W power and 20Hz frequency for 20 seconds on teeth undergoing erosive challenge by acidic drinks. Although this observation agrees with the present findings, Dionysopoulos et al. [20] used bovine teeth and 0.25W laser in their study. Da Silva et al. [12] reported that Er,Cr:YSGG laser irradiation with 5.7J/cm^2 power and 20Hz frequency, as well as fluoride treatment, ceased dental erosion progression, which disagrees with our findings. The discrepant findings of the two studies might be due to different pH cycling protocols and specimen characteristics. Human third molars were used in the present study while Da Silva et al. [12] used bovine teeth. In their study, bovine teeth were exposed to 3% citric acid for 5 minutes and then immersed in artificial saliva for 1 hour; this procedure was repeated 4 times daily for 5 days. In the present study, on the other hand, the specimens' exposure to lemonade for 2 minutes was followed by 12 hours of immersion in artificial saliva; this procedure was repeated 2 times a day for 3 days.

Geraldo-Martins et al. [21] presented evidence of optimal efficacy of Er,Cr:YSGG laser irradiation with 0.25W power and 4.48J/cm^2 fluence for 20 seconds without air and water to increase enamel resistance. Despite the alignment of this result with our findings, the discrepancy is that the impact of laser irradiation combined with eggshell solution, and eggshell solution alone was also examined in the current study, along with a different pH cycling method.

The current study had some limitations, including an in vitro design and utilizing a laser with a specific type and wavelength. This study evaluated enamel resistance to erosion in vitro. Future studies are recommended to assess this effect in vivo or in situ. Future investigations are also needed on the effectiveness of other laser parameters, as well as on the combination of laser and eggshell solution to study their impact on erosive lesions. Our prepared eggshell powder can also be elaborated as a toothpaste to apply on erosive wounds. The present study assessed enamel resistance to erosion conferred by remineralizing agents, similar to some other studies [22,23], without electron microscopy. Further studies are also recommended to assess enamel resistance to erosion following the application of laser and eggshell solution using electron microscopy.

CONCLUSION

According to the present results and considering the limitations of this in vitro study, an increase in microhardness was observed after remineralization only in the fluoride group, and its decrease was not significant after demineralization. Therefore, although the changes in fluoride group were not significant, fluoride was more effective than artificial saliva, Er,Cr:YSGG laser, eggshell solution, and the combination of eggshell solution and Er,Cr:YSGG laser in enhancing enamel resistance to lemonade-induced erosion. Additionally, it appears that the combination of Er,Cr:YSGG laser and eggshell solution provided better acid resistance than either treatment alone.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interests to declare.

GENERATIVE AI IN SCIENTIFIC WRITING

None declared.

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