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Effects of Actimins Containing Toothpaste and Er,Cr:YSGG Laser on Remineralization of Primary Enamel Lesions

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Abstract

Introduction: Currently, the main focus of caries control measures is on methods for early detection and non-invasive treatment of incipient lesions. The present study aimed to compare the efficacy of a toothpaste containing Actimins and an Er,Cr:YSGG laser on the microhardness of demineralized enamel.

Methods: Forty maxillary and mandibular primary posterior teeth with sound buccal or lingual surfaces were evaluated in this in vitro study. After measuring their baseline microhardness, the teeth were immersed in a demineralizing solution for 48 hours, and their microhardness was measured again. The teeth were then randomly divided into 5 groups of negative control (artificial saliva), positive control [1.23% acidulated phosphate fluoride (APF)], Actimins, Er,Cr:YSGG laser (0.75 W power, 20 Hz frequency, 60% water, 40% air, and 60 s pulse duration), and Actimins plus Er,Cr:YSGG laser, and underwent microhardness measurement for the third time after the intervention. The microhardness data were compared by an ANOVA and a Bonferroni test with the significance level set at 0.05.

Results: The toothpaste containing 5% Actimins and the Er,Cr:YSGG laser significantly enhanced the remineralization of primary enamel incipient lesions. The lowest mean microhardness at baseline, after demineralization, and after remineralization was noted in the artificial saliva group. No significant difference was found in microhardness between the groups at baseline or after demineralization ($P > 0.05$).

Conclusion: According to the results, the toothpaste containing 5% Actimins and the Er,Cr:YSGG laser significantly enhanced the remineralization of primary enamel incipient lesions.

Keywords: Demineralization, Remineralization, Microhardness, Actimins, Incipient enamel lesions

Introduction

Dental caries is a multifactorial condition.[1] Carious lesions are often removed by a bur; nonetheless, the main focus is currently on non-invasive treatment of carious lesions. Non-invasive treatment of incipient carious lesions includes their remineralization by using a number of products proposed for this purpose, such as fluoride, NovaMin, tricalcium phosphate (TCP), and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP).[2]

Fluoride is among the most widely known remineralizing agents, which can prevent demineralization and improve remineralization of incipient caries. However, it has relative potential for the prevention of bacterial invasion and the enhancement of remineralization.[1] Also, it has a limited effect on the prevention of demineralization due to the gradual dissolution of the formed calcium fluoride layer following the consumption of acidic foods and drinks, which is another drawback. Thus, finding a durable method for the enhancement of enamel resistance and the subsequent promotion of oral health appears to be imperative.[3]

Bioactive glass is a unique biomaterial capable of regeneration of bone and remineralization of dentin and incipient enamel lesions.[4,5] Bioactive glass is a biomimetic remineralizing agent, and it can also affect the cell signals to improve tissue healing. It is composed of sodium, phosphorous, calcium, and silica, which are naturally found in the human body. When exposed to saliva and water, bioactive glass releases these ions, causing enamel surface remineralization.

They have been shown to be capable of inducing in vivo

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responses, including osteoconduction, bone adhesion via ion release, and the potential for apatite layer formation, and they have been reported to induce the mineralization of dentin surfaces. Another approach is to remineralize early enamel lesions using synthetic hydroxyapatite minerals that are believed to be similar to natural enamel. The purpose of these synthetic minerals is to strengthen partially degraded hydroxyapatite crystals in enamel through the accumulation and incorporation of calcium and phosphate ions.[5]

Actimins® is a novel dental desensitizer composed of a bioactive nano-crystal powder that can occlude the dentinal tubules and decrease tooth hypersensitivity. Enamel remineralization, prevention of demineralization and caries, and treatment of incipient caries in children are among its other advantages.[6] It is mainly composed of sodium, calcium, phosphorous, and silicon, and its mechanism of action is through the occlusion and obstruction of dentinal tubules and restoration through the remineralization and improvement of the oral environment through the induction of osmotic changes by releasing sodium and calcium ions.

It has been reported that the irradiation of lasers can also alter enamel composition, enhance its acid resistance, and prevent caries as such. Laser irradiation causes chemical and structural changes, increases fluoride uptake, enhances remineralization, and decreases the risk of caries.

The Er,Cr:YSGG laser is used in a 78.2 µm wavelength for dental procedures such as caries removal, cavity preparation, root canal treatment, and surgical procedures. This particular wavelength is optimally absorbed by the OH- groups of hydroxyapatite crystals, raises the surface temperature to 800°C, and subsequently causes crystallographic changes in the enamel without evaporation or melting of the carbonated hydroxyapatites [7]. Also, the Er,Cr:YSGG laser is superior to the Er:YAG laser due to its higher absorption by hydroxyapatite, deeper penetration, and lower erosion.[8] Some studies compared the efficacy of lasers with remineralizing agents for the remineralization of erosive enamel lesions or incipient enamel caries and reported the positive efficacy of lasers for this purpose.[8,9,10] However, some other studies did not notice any significant effect.[6,7]

Considering the shortcomings of fluoride and the advantages of the suggested alternatives for the remineralization of demineralized enamel, comparison of their efficacy with fluoride seems logical. Thus, this study aimed to compare the effects of a toothpaste containing Actimins and the Er:Cr:YSGG laser on the microhardness of demineralized enamel.

Materials and Methods

This in vitro study was ethically approved by the ethics committee of Shahed University of Medical Sciences (IR.SHAHED.REC.1400.131). Forty extracted primary maxillary and mandibular molar teeth were collected from dental clinics in Tehran. All the collected teeth had been extracted due to non-restorable caries. The sample size was calculated according to the following formula:

$$n = (Z\alpha/2 + Z\beta)2 * (p1(1-p1) + p2(1-p2)) / (p1-p2)2$$

Primary molar teeth with sound buccal or lingual surfaces with no caries, hypoplasia, restoration, crack, or fracture were included in this study.

Sampling was performed in two steps. First, the teeth were collected by convenience sampling, and then they were randomly assigned to 5 groups. The teeth were first mounted in auto-polymerizing acrylic resin. To standardize the depth and width of incipient carious lesions, the enamel surface was first polished with 600-, 800-, and 2400-grit silicon carbide abrasive papers, and then with aluminum oxide discs. Also, the area around the exposure site was coated with acid-resistant nail varnish, leaving an exposed window at the center measuring 4 × 4 mm.[2,6]

Next, the microhardness of all teeth was measured by a Vickers hardness tester (Bareiss prufgratebau GmbH, Germany) by applying a 100-g load for 5 seconds perpendicular to the enamel block. Then, to induce incipient enamel lesions, the teeth were immersed in a demineralizing solution containing 2.2 mM calcium chloride, 2.2 mM monosodium phosphate, and 0.05 M lactic acid at 37C for 48 hours. Subsequently, they underwent microhardness testing again, and afterwards, they were randomly assigned to 5 groups using a list of random numbers generated by SPSS.

Group 1 (control): The teeth were immersed in artificial saliva composed of 2.2 g/L gastric lucin, 0.381 g/L sodium chloride, 0.213 g/L calcium chloride, 0.738 g/L potassium hydrogen phosphate, and 1.114 g/L potassium chloride. One liter of artificial saliva was prepared at the Dental Material Research Center of Tehran University of Medical Sciences.

Group 2 (positive control): The teeth were exposed to 1.23% acidulated phosphate fluoride (APF) for 4 minutes/day for 14 days.

Group 3 (Actimins): The teeth were exposed to 5% Actimins gel in the form of a toothpaste for 5 minutes daily for a total period of 14 days. Actimins was composed of 95% PEG 400, glycerol, silicon dioxide, and 5% calcium sodium phosphosilicate active substance. This toothpaste was synthesized at the School of Bioscience of Tarbyat Modarres Hospital.

Group 4 (Er,Cr:YSGG laser): The teeth were subjected to Er,Cr:YSGG laser irradiation (Biolase Technology, Inc., USA) with 0.75 W power, 20 Hz frequency, 60% water, 40% air, 60 s pulse duration, 0.75 output energy, 16 mm area, 7.5 J/cm^2 pulse energy, and 281.25J/cm2 total

Table 1. Mean and standard deviation of the microhardness of the groups at different time points (n = 8 in each group)

Group	Remineralization	Demineralization	Baseline
1. artificial saliva	192.50 (SD = 42.90)	192.87 (SD = 43.69)	381.00 (SD = 47.35)
2. 1.23% APF	262.50 (SD = 14.01)	199.37 (SD = 10.07)	387.87 (SD = 48.99)
3. 5% Actimins	235.50 (SD = 17.14)	196.37 (SD = 17.51)	390.12 (SD = 41.41)
4. (Er,Cr:YSGG)	242.25 (SD = 8.82)	194.37 (SD = 8.65)	
5. Er,Cr:YSGG + Actimins	256.50 (SD = 13.75)	197.25 (SD = 19.73)	389.50 (SD = 36.78)
Total	237.85 (SD = 33.00)	196.05 (SD = 22.46)	388.65 (SD = 41.40)

SD: Standard deviation

energy (Table 4).

Group 5 (Er,Cr:YSGG laser plus 5% Actimins): The teeth were subjected to Er,Cr:YSGG laser irradiation with 0.75 W power, 20 Hz frequency, 60% water, 40% air, and 60 s pulse duration and were then treated with Actimins twice a day each time for 2 minutes for a total of 14 days.

After 28 days, the microhardness of the teeth was measured again. All the teeth were stored in artificial saliva after each treatment until microhardness testing.

The Shapiro-Wilk test showed normal distribution of microhardness data. Thus, an ANOVA and a Bonferroni test were used for data comparison with the significance level set at 0.05.

Results

The microhardness of the groups at baseline, after demineralization, and after394.75 (SD = 41.55) Remineralization ranged from 381.00 to 394.75 HV, 192.87 to 199.37 HV, and 192.50 to 262.50 HV, respectively. Also, the mean microhardness of the groups at baseline, after demineralization, and after remineralization was 388.65 ± 41.40 HV, 196.05 ± 22.46 HV, and 237.85 ± 33.00 HV, respectively (Table 1).

The highest mean microhardness at baseline, after demineralization, and after remineralization was noted in the Er,Cr:YSGG laser, 1.23% APF, and 1.23% APF groups, respectively. The lowest mean microhardness at baseline, after demineralization, and after remineralization was recorded in the artificial saliva group (Table 1).

There were no significant differences between the groups in microhardness at baseline ($P = 0.980$) or after demineralization ($P = 0.984$). However, there was a significant difference in microhardness between the groups following remineralization ($P < 0.001$).

Furthermore, ANOVA revealed a large effect size ($\eta^2 = 0.44$) after remineralization, indicating that approximately 44% of the variance in microhardness values can be attributed to differences between the groups. According to commonly accepted benchmarks (small = 0.01, medium = 0.06, large = 0.14), this represents a substantial effect, suggesting that remineralization had a considerable impact.

In contrast, at baseline and after demineralization, the η^2 values were small and the results were not statistically significant, implying that differences between the groups during these phases were minimal and not meaningful (Table 2).

Pairwise comparisons were performed by the Bonferroni test (Table 3).

As shown in Table 3, significant differences were noted in microhardness between artificial saliva and 1.23% APF ($P < 0.001$), artificial saliva and 5% Actimins ($P = 0.006$), artificial saliva and Er,Cr:YSGG laser ($P = 0.001$), and artificial saliva and Er,Cr:YSGG laser + 5% Actimins ($P < 0.001$) after remineralization.

Discussion

The present results showed that 5% Actimins toothpaste and Er,Cr:YSGG laser irradiation significantly enhanced the remineralization of primary enamel incipient lesions.

Actimins is a bioactive nano-crystal powder composed of sodium, calcium, phosphorous, and silicone, which can remineralize the enamel.[11] Actimins® (calcium sodium phosphosilicate) is composed of elements that occur naturally in the body (Ca, Na, Si, P, and O). When exposed to an aqueous environment, Actimins® undergoes a rapid surface reaction that leads to the rapid release of soluble ionic species (such as calcium and phosphate ions) to form polycrystalline hydroxycarbonate apatite deposition inside and onto dentinal tubules, which effectively occludes the dentinal tubules and blocks hydrodynamic flow to eliminate tooth hypersensitivity. The formed hydroxycarbonate apatite layers on the tooth surface are similar to the composition and structure of natural tooth mineral, making the tooth stronger and healthier.[6]

Laser irradiation can cause physical fusion of enamel prisms. It can also decrease enamel permeability and dissolution by melting, and cause reattachment and re-crystallization of enamel crystals. The reduction of enamel dissolution can be due to crystallographic changes of the enamel,[12] which occur due to heat generation by laser, decreasing the carbonate and organic material content (water and protein) and increasing the size of crystallites and formation of tri-calcium phosphate, tetra-calcium phosphate, and hydroxyl ions. In total, these changes enhance the overall enamel resistance to acid attacks.[13-16] The elimination of enamel matrix organic materials causes the obstruction of intra- and inter-prismatic spaces, which

Table 2. Results of ANOVA regarding the effect of treatments on microhardness during the study period (n = 40)

		Sum of Squares	Df	Mean Square	F	Sig.	Eta-squared	95% Confidence Interval	
								Lower	Upper
Baseline	Between-group	793.850	4	198.462	.105	.980	0.012	0.000	0.000
	Within-group	66069.250	35	1887.693					
	Total	66863.100	39						
Demineralization	Between-group	203.900	4	50.975	.092	.984	0.09	0.000	0.21
	Within-group	19472.000	35	556.343					
	Total	19675.900	39						
Remineralization	Between-group	24295.600	4	6073.900	11.687	.000	0.44	0.13	0.57
	Within-group	18189.500	35	519.700					
	Total	42485.100	39						

Table 3. Pairwise comparisons of the microhardness of the groups after remineralization (n = 40)

Groups		Mean Difference	Std. error	95% CI of OR	*P* value
C-	C+	-70	11.39	-104.15 to -35.84	<0.001
C-	A	-43	11.39	-77.15 to -8.84	0.006
C-	L	-49.75	11.39	-83.90 to -15.59	0.001
C-	A+L	-64	11.39	-98.15 to -29.84	<0.001
C+	A	27	11.39	-7.15 to 64.15	0.235
C+	L	20.25	11.39	-13.90 to 54.40	0.843
C+	A+L	6	11.39	-28.15 to 40.15	1.000
A	L	-6.75	11.39	-40.90 to 27.40	1.000
A	A+L	-21	11.39	-55.15 to 13.15	0.739
L	A+L	-14.25	11.39	-48.40 to 19.90	1.000

C-: Artificial saliva; C+: 1.23% APF, A: 5% Actimins; L: Er,Cr:YSGG laser; A+L: 5% Actimins+Er,Cr:YSGG laser

Table 4. Laser parameters

Power	Frequency	Water	Air	Pulse Duration
0.75 W	20 Hz	60%	40%	60 s

decreases ionic dispersion, and delays demineralization.[17]

The present results were in agreement with the findings of Yilmaz et al[17]and Sadabadi et al,[10] but they were in contrast to the results of Ana et al[13] Azevedo et al[18] and Molaasadollah et al.[7] This difference may be due to variations in laser power and pulse since low laser power is not strong enough to cause sufficient positive changes in the composition and orientation of hydroxyapatite crystals. On the other hand, using very high powers of the laser can change and irreversibly degrade the hydroxyapatite crystals, preventing optimal remineralization of enamel. Moreover, some previous studies evaluated permanent teeth, which may yield different results compared to primary teeth used in the present study. The reason is that permanent teeth have a higher mineral (hydroxyapatite) content, and thus they undergo different crystallographic changes in the enamel following laser irradiation compared to primary teeth, which can affect their remineralization potential.[13,19]

Soltanimehr et al. evaluated the combined effect of bioactive glass [TCP-5% sodium fluoride (fTCP) and CPP-ACP] and diode and CO2 lasers on the remineralization of primary enamel. They showed that diode and CO2 lasers had no significant efficacy for primary enamel remineralization. On the other hand, the application of bioactive glasses (fTCP and CPP-ACP) increased the microhardness and remineralization of primary teeth.[6] There are two theories explaining the laser's mechanism of action. The first theory states that lasers cause the accumulation of calcium, phosphate, and fluoride ions in cracks and porosities. The second theory explains that fluoride penetrates into hydroxyapatite crystals and forms fluorapatite mediated by laser-induced heat. According to the first theory, the bond that forms between the tooth structure and ions is weak, while according to the second theory, a strong bond forms between the ions and tooth structure.[20] Also, the irradiation of laser melts dentinal tubules and obstructs them. Moreover, water, organic compounds, and carbonate ions are released from hydroxyapatite due to the generated heat and expedite the remineralization process.[21]

The CO2 laser (10600 nm wavelength) has the highest absorption in tissues with a high water content, such as soft tissue. Also, the diode laser (819 nm wavelength) has the highest absorption in pigmented soft tissues and congested tissues, whereas the Er,Cr:YSGG laser (2780 nm wavelength) has the highest absorption in tissues with a high water content and hydroxyapatite.[22,23] Thus, the Er,Cr:YSGG laser has a higher capacity than CO2 and diode lasers to affect enamel hydroxyapatite crystals and increase the chance of remineralization.

Haghgoo et al[24] showed that CPP-ACP and TCP enhanced the remineralization of erosive lesions similar to Actimins and increased enamel microhardness. However, the difference in microhardness was not significant between the CPP-ACP and TCP groups. Similar to Actimins, CPP-ACP and TCP can stabilize calcium and phosphorous ions on the tooth surface and increase the microhardness and remineralization of enamel.[24]

On the other hand, according to a study by Tavassoli et al[25] on fissure sealants, microhardness and remineralization potential may increase by using a higher concentration of TCP (5% compared to 1%). Consistent with the present results, evidence shows that nano-hydroxyapatite toothpastes and NovaMin are effective for the remineralization of primary enamel incipient caries. This efficient remineralization may be explained by the replacement of sodium ions with hydrogen ions in the formulation of calcium sodium phosphosilicate bioactive glass, which raises the pH and causes the deposition of calcium and phosphate ions on the tooth surface for the formation of a superficial layer saturated with calcium phosphate.[26]

This study assessed the efficacy of Actimins and Er,Cr:YSGG laser for the remineralization of incipient enamel lesions. Future studies are recommended on the effects of Er,Cr:YSGG laser and 5% Actimins on the remineralization of primary enamel incipient caries using other methods such as electron microscopy. Also, the short-term effects of remineralizing agents were evaluated in the present study; the assessment of the long-term effects of such interventions is recommended to obtain more accurate results.

The present study evaluated the in vitro effects of remineralizing agents; thus, their effects should be further evaluated in the clinical setting. Also, demineralization progresses more rapidly in primary teeth compared to permanent teeth.[17] Future studies are required to compare the effects of Actimins and lasers in the restoration of incipient lesions of primary and permanent teeth.

Conclusion

According to the results, the toothpaste containing 5% Actimins and the Er,Cr:YSGG laser significantly enhanced the remineralization of primary enamel incipient lesions.

Authors' Contribution

Conceptualization: Alireza Aghajanian
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Investigation: Alireza Aghajanian
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Writing–original draft: Roza Haghgoo
Writing–review editing: Majid Mehran

Competing Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical Approval

This research was approved by the research ethics committee of Shahed University of Tehran (IR.SHAHED.REC. 1400.131) and there were not any ethical considerations.

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