The effect of different re-mineralizing agents and diode laser irradiation on the microhardness of primary molar enamel: An *in vitro* study

Zahra Bahrololoomi [1], Fatemeh Zarebidoki [2], Nasrin Mostafalu [3*]

1: Associated Professor, Social Determinants of Oral Health Research Center, Department of Pedodontics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2: Assistant, Department of Pedodontics, Shahid Sadoughi University of Medical Sciences, Faculty of Dentistry, Yazd, Iran
3: Assistant professor, Department of Pedodontics, Semnan University of Medical Science, Semnan, Iran

Background and Aims: Dental caries is a global concern and different materials and methods were proposed for its prevention.
The aim of this study was evaluation of the effect of different demineralizing agents with and without diode laser radiation on the microhardness of primary molars enamel.
Materials and Methods: 48 primary molar teeth were used in this study. The enamel blocks were prepared. Primary microhardness values were also measured. All samples were demineralized, then demineralization was approved by a DIAGNOdent pen. The remineralization was done into the buccal and lingual surfaces of the samples as follows:
Group 1: CPP-ACP, Group 2: CPP-ACPF, Group 3: MI varnish, Group 4: NaF varnish. The lingual surfaces received diode laser irradiation. Finally, a secondary microhardness test was performed on all samples. Statistical analyses were done and a P-value less than 0.05 was considered to be statistically significant.
Results: A comparison between the primary and the secondary microhardness values showed significant differences ($P = 0.003$). The highest secondary microhardness values belonged to the MI varnish +laser group.
Conclusion: Application of the agents, containing the combination of Ca, P, and F, was significantly better than others in increasing the microhardness of the enamel. Although the application of diode laser increased the hardness of the enamel.

Key words: Diode laser • Microhardness • Primary molar • Demineralizing agent

Introduction:

Dental caries is the most common chronic disease among children and it is 5 and 7 times more prevalent than Asthma and Hay Fever, respectively [1)]. Caries progression is a dynamic process caused by a misbalance between the remineralization or the demineralization factors [2)] even if other different factors may affect the susceptibility of teeth to caries [1, 3)].

The first sign of caries is white spot lesion while the enamel surface is intact and the subsurface layers are demineralized and will be infected with cavities if the treatment is delayed [4, 5)]. The white spot lesions have a lower hardness value in comparison to the intact enamel [3)]. These lesions, if coated with demineralizing agents, have demineralizing potential and, consequently, would also be more resistant against acidic challenges [3)]. Different methods and materials were introduced for the improvement of remineralization and topical fluoride application is the most used current method for the control of primary lesions. However, due to the rise in fluorosis risk from fluoride swallowing, many investigations were done to introduce new material and methods [6, 7)].

Recently, remineralization technology based on calcium phosphate (Casein Phospho Peptide Amorphous Calcium Phosphate or CPP-ACP) yield interesting results on remineralization of the primary lesions [8)]. CPP is a car-

**Addressee for Correspondence:*
Nasrin Mostafalu
Dept. of Pedodontics ,Faculty of Dentistry, Daheye Fajr BLV, Imam Ave,Po Box: 89195/165 Yazd, IRAN
Tel: +98-351-6255881 Fax: +98-351-6250344E
E-mail: nasrin134314@yahoo.com

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rier that localizes the calcium phosphate phase on the surface of the teeth, so remineralization is accelerated [9]. With due attention to the synergic effect of CPP-ACP with fluoride, a new composition called MI paste plus (CPP-ACPF) was introduced that seemed to be effective in controlling caries [10]. The MI varnish is a product with calcium, phosphate, and fluoride in varnish form.

Another new different method is laser irradiation [11] which is able to decrease enamel permeability and solubility so increasing the remineralization process [12] and, additionally, it may decrease the amount of Streptococcus mutans bacteria in the mouth, so playing a key role in dental caries prevention [13]. Melting and re-crystallization of surface enamel and change in enamel morphology, decreasing in water and carbonate content in teeth, and decreasing enamel solubility with calcium diphosphate monoxide following laser irradiation may cause higher resistance in enamel against caries [14]. In spite of the great application of laser in dentistry, its useful effect as an adjunct method of remineralization has not been studied in detail [15].

Studies about laser radiation with fluoride therapy showed lower morphologic changes and enamel cracks on the enamel surfaces [16, 17]. Subramanian and Ulkur proved the beneficial effect of the laser with demineralizing materials on remineralization of enamel [9, 18, 19]. The diode laser is a small and portable device with easy application and lower cost that makes it preferable to other technologies. Also, we have little information about its cariostatic effects [20].

In vitro evaluation of de-remineralization was done using different methods. Vickers microhardness test is a simple, non-destructive and rapid method for the evaluation of the enamel structure [6].

Due to the lack of sufficient studies done on these subjects, especially primary dentitions, this study were designed to evaluate the effect of different demineralizing materials with diode laser radiation on the microhardness of primary molar enamel.

Materials and methods

At this experimental study, 48 primary molar teeth with intact buccal and lingual surfaces were selected. The exclusion criteria were the detectable defects on enamel surfaces and also presence of carious lesions, hypoplastic, and white spot lesions. The teeth were immersed in Chloramine T 0.5% for 7 days and then kept in distilled water at room temperature [21, 22]. The roots were dissected using high-speed handpiece and the crowns were mesiodistally divided into the buccal and lingual sections. Enamel slabs with 4 × 2 × 2 mm dimensions were prepared from each block and embedded in acrylic resin and labelled from 1 to 48. They were randomly divided into four groups with each section comprising 12 primary molars. Each specimen had two subgroups: a. buccal surface and b. lingual surface. The slabs were polished, respectively, with 300, 600 and 1200 grit silicon carbide papers and with slurry of alumina to achieve a flat enamel surface [21, 22].

The primary microhardness values were measured using Vickers method (Microhardness tester, FM700 series, Future-tech corp., Japan)

The hardness values were measured thrice and the mean value was considered as a baseline. (Test load = 50g/f, dwell time = 5 seconds). Then, the demineralization process was done in the chemistry department. All the samples were stored in a 50ml demineralization solution for 48 hours at 37°C. The demineralization solution consisted of [4]:

2.2Mm $CaCl_2.2H_2O$ (Merck KGa A, Germany)
2.2Mm $NaH_2PO_4.7H_2O$ (Merck KGa A, Germany)
0.05M Lactic acid (Merck KGa A, Germany)

Then, the specimens were again evaluated using a DIAGNOdent pen (Kavo, Germany) to assure the artificial subsurface caries were obtained. After that, the remineralization process was conducted to each group:

Group 1: CPP-ACP (GC tooth mousse, Tokyo, Japan)
Group 2: CPP-ACPF (MI paste plus, Tokyo, Japan)
Group 3: CPP-ACPF varnish (MI varnish, GC Corporation, Tokyo, Japan)
Group 4: NaF varnish (Flurilaq Pascal International Inc,USA.)

The remineralizing agents were rubbed on the enamel surfaces for four minutes and then immersed in artificial saliva [23]. The artificial saliva had the following component: Gastric musin, NaCl, $CaCl_2.2H_2O$, $K_2HPO_4.3H_2O$, KCl [24].

The remineralizing agents were applied every day. This process was continued for seven days. After that, the lingual surfaces from each of the groups were put through laser radiation. The laser radiation was done in pulsed mode as proposed by several Authors [21, 25]. Diode laser (ARC laser, FOX, Germany) was used (Wavelength = 980 nm, Frequency = 15 Hz, Power = 5W, Beam diameter = 600 µm, Pulse duration and Pulse interval = 30 ms, Power density= 62/5 W/cm^2 and Energy= 150 J). Distance from the samples was 5mm using a custom-made holder. Exposure of enamel surfaces was in scanning mode attending to the suggestion of several works [21, 26].

Finally, a secondary microhardness test was conducted to all the samples and the differences were calculated.

The data analyses were done using SPSS version 23. The quantitative data comparisons were accomplished by performing the Kolmogorov-Smirnov test. In order to compare between the two groups, the Kruskal-Wallis test was done. The Wilcoxon test was done to compare between the before and after stages of each group. A Post hoc analysis and the Dan test were executed to evaluate the non-parametric comparisons.

Results

The mean value of the primary microhardness numbers (MHN1) was calculated. The difference of MHN1 among the groups was not statistically significant (P = 0.158). **(Table 1)**

Comparison between the primary and the secondary microhardness numbers showed significant differences (P = 0.003). **(Table 1)**

The highest secondary microhardness number (MHN2) was observed in the MI varnish + laser group. The groups were arranged in the following order according to the increase in microhardness:

MI varnish +laser > MI varnish > MI paste > CPP-ACP > CPP-ACP +laser > MI paste + laser > NaF varnish + laser > NaF varnish

The MI varnish and MI paste groups with or without laser radiation showed statistically significantly higher microhardness increase than that in the NaF varnish group. But the other groups did not register significant difference among themselves.

The mean value of MHN1 in subtype a (buccal surface that received only remineralization treatment) and subtype b (lingual surface that received remineralization and laser radiation) were compared and it did not show significant difference (P = 0.767). **Table 2** shows a com-

Table 1 : Comparison of mean microhardness numbers in different groups

Groups	N	MHN1	Inter-quarter range for MHN1**	MHN2	Inter-quarter range for MHN2
CPP-ACP*	12	341.875	109.5	393.742	112.6
MI paste	12	385.142	79.9	439.358	58.4
MI varnish	12	389.842	38.9	444.458	22.1
NaF varnish	12	368.1	62.8	380.6	44.8
CPP – ACP + laser	12	340.592	101.2	388.567	158.8
MI paste + laser	12	383.442	86.4	415.250	135.8
MI varnish + laser	12	388.95	36.4	449.058	36.7
NaF varnish + laser	12	367.658	61.9	390.675	47.7

P-value of MHN1 = 0.158, P-value of MHN2 = 0.003
* CPP – ACP: Casein phosphor peptide amorphous calcium phosphate
**MHN: Microhardness number

Table 2 : Comparison of MHN2 in different groups with and without laser radiation

Groups	N	Mean MHN2	Inter-quarter range	P-value
Subtype a: **CPP- ACP*** **MI paste** **MI varnish** **NaF varnish**	48	414.5	62.5	0.97
Subtype b: **CPP – ACP + laser** **MI paste + laser** **MI varnish + laser** **NaF varnish + laser**	48	410.8	67.4	0.97

*CPP - ACP: Casein phosphor peptide amorphous calcium phosphate parison among these groups according to their value of MHN2 that showed no statistical difference to (P = 0.97).

Discussion

This study was designed for evaluation of different remineralizing agents and their efficacy with diode laser radiation on the microhardness of primary molar enamel. Because of a lower percentage of mineral composition and dentin thickness, primary dentition has a higher risk of dental caries. So, the application of remineralizing agents is more important [9)]. Dental caries is a dynamic process with a low progression speed and the preventive treatment for this can be achieved by using different remineralizing materials [27)].

Surface microhardness test is a suitable method for the evaluation of fine microstructure, non-homogenous and susceptible to crack surfaces. So, we used this method to compare the efficacy of different materials on enamel surface [28)].

All the remineralizing materials increased the mean microhardness values of primary molar enamel. This increase was more significant in MI varnish and MI paste groups, which is in agreement with the results of other studies [29, 30)]. Different studies proved the synergic effect of fluoride and CPP-ACP [4, 10, 31)]. All remineralizing agents have significantly increased the microhardness of primary molar enamel in comparison to the base values, however, materials based on calcium, phosphate, and fluoride yielded better results.

One of the interesting results of this study is the significantly higher enamel microhardness values in the MI paste group than the CPP-ACP group, which is consistent with the result of the Patil et al. study [4)].

In Shetty's study, the application of CPP-ACPF was significantly better than the CPP-ACP and NaF varnish, which is consistent with our results [10)]. CPP-ACPF contains calcium, phosphate, and 900ppm fluoride and is commercially available as MI paste plus. Penetration of this nanocomplex into the dental plaque allows Ca, P, F precipitation on the surface of the teeth. Consequently, it also increases the microhardness value of the enamel [31)].

The result of this study and the others is in contrast to Lata's study. Lata showed that the fluoride varnish was better in remineralizing primary caries than CPP-ACP. The combination of fluoride and CPP-ACP has no advantage in comparison to fluoride alone. Moreover, none of these materials had the potential for subsurface caries remineralization [3)]. In the study, a 5-day PH cycling was done after demineralization and remineralization that might have affected the results. They used permanent teeth for their studies. The application of cross-sectional microhardness test in Lata's study may be the reason behind these different results. To explain why MI varnish is more effective than MI paste in increasing enamel microhardness, we assume that the application of Ca, P, and F in varnish form can increase the time of contact with enamel and also increase microhardness consequently.

The results of this study are similar with the results of the studies done by Patil, Subramaniam, Gangrande, Bahrololoomi, and Asl-Aminabadi and other investigators [6, 9, 21, 23, 24)].

Although laser radiation had increased the microhardness of the enamel, this improvement was not significant in comparison with the buccal segment in each group that only received remineralization treatment. With due attention to the results of this study, laser application in order to increase the microhardness of primary molar enamel is not justified.

In this study, pulse mode laser radiation was applied, but the use of a continuous mode of radiation might have improved the results.

In the present study, the mean increase in microhardness in the CPP-ACP group is more than that in CPP-ACP + laser group. However, this advantage is not significant. Asl-Aminabadi et al. achieved the same results in their studies. They concluded that most of the composition of calcium, phosphate was in the CPP-ACP + Nd:YAG laser group. While the surface microhardness value of the CPP-ACP group was more than that of the CPP-ACP + laser group. The Nd:YAG laser application with high power may be responsible for creating surface defects and enamel cracks and, consequently, lowering microhardness in the CPP-ACP + laser group in comparison to the CPP-ACP group.

Subramaniam et al. showed that Er: Cr: YSGG laser application with CPP-ACP significantly increased the microhardness of primary molar. However, they used the Brinell test for evaluating the microhardness and the demineralization was done by applying citric acid 1% for 30 minutes. A different demineralization time period and method and the use of different type of laser may explain the different results of this study. Moreover, they had five days of remineralization. That additional remineralization time may affect the efficacy of the remineralizing materials in our study.

In this study, the application of the NaF varnish increased the microhardness. However, in three samples of the NaF varnish group and two samples of the NaF varnish + laser group, the microhardness values didn't reach the baseline values. Seven days of remineralization may be insufficient for NaF varnish. The mean microhardness values of the NaF varnish and NaF varnish + laser groups were significantly lower than that of the MI varnish and MI paste groups. The occurrence of a higher microhardness value following the application of NaF varnish with or without laser irradiation adheres to the Bahrololoomi et al. study that reported that the fluoride varnish and laser can increase the hardness of primary teeth [21)].

The study conducted by Azevedol et al yielded the same results. They concluded that the APF and APF + laser groups have experienced fewer changes in the microhardness value following demineralization and the depth of demineralization in all remineralizing groups was lower than that of the control groups. They showed that the Nd:YAG laser with remineralizing is not more effective in stopping demineralization than the fluoride therapy [32].

According to the results of this study, the application of products with calcium, phosphate, and fluoride are suggested. Hence, MI varnish is suggested for use in office and MI paste is preferable for use at home. In order to keep infants away from swallowing fluoride, it is better to use MI varnish with CPP-ACP (GC tooth mousse) at home while older children with enamel hypoplasia and MIH can benefit from the use of MI varnish with a daily application of MI paste at home.

This study was designed *in vitro*. Comparison of these methods and materials *ex vivo* or the provision of a longer period for remineralization (more than seven days) and the PH cycling and its evaluation using different methods such as the SEM, the Brinell test, and the Knoop hardness test are suggested.

Conclusion

Application of the agents, containing the combination of Ca, P, and F, was significantly better than others in increasing the microhardness of the enamel. Although the application of diode laser increased the hardness of the enamel.

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Conflict of interest statement
No conflict of interest was declared