

Comparison of traditional and novel remineralization agents: A laser fluorescence study

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Original Article

Abstract

BACKGROUND AND AIM: The first clinical symptom of tooth decay is the appearance of a white spot lesion (WSL) on the tooth surface. This study is performed with the aim to evaluate the efficacy of six different remineralization agents in vitro with the DIAGNOdent laser fluorescence method.

METHODS: In this study, 44 extracted, impacted human wisdom teeth (88 specimens) without caries were used to obtain standard demineralized enamel surfaces. The teeth were divided into eight groups consisting of two control (positive and negative) and six experimental groups [Fluoride (F) gel, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) cream, CPPACP + F cream, NovaMin + F toothpaste, Xylitol-Hydroxyapatite + F cream, and Ozone + F]. De/re-mineralization solutions were applied with a pH-cycle approach to areas of 3 × 3 mm on the buccal/lingual surfaces of the teeth for 9 days. The remineralization agents were applied to the demineralized areas and the samples were then kept in artificial saliva. The DIAGNOdent measurements were performed before and after the application of the remineralization agents. The Wilcoxon and Kruskal-Wallis tests were used for analysis of the data.

RESULTS: The mean scores obtained after the DIAGNOdent measurements were 3.82 and 6.91 in the negative-control and positive-control groups, respectively. There were significant differences in the scores before and after the remineralization procedure in all experimental groups ($P < 0.050$). When the mean differences before and after treatment were compared between the groups, CPP-ACP + F provided significantly more remineralization than other experimental groups ($P < 0.050$).

CONCLUSION: Remineralization of demineralized areas was achieved in all experimental groups. The highest degree of remineralization according to the DIAGNOdent scores before and after the procedure was observed in the CPP-ACP + F group.

KEYWORDS: Tooth Remineralization; Lasers; Fluorescence; Xylitol; Ozone

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Tooth decay is one of the most common and preventable diseases of childhood. The process of caries formation is a cycle of remineralization and demineralization, the various stages of which may be reversible or irreversible.¹ The first clinical manifestation of tooth decay is the appearance of a white spot lesion (WSL) on the tooth surface, which is thought to be the initial stage of enamel demineralization.² If the demineralization process continues, the initial lesion may progress and lead to cavitation.³

A WSL is the earliest macroscopic sign of enamel caries. Typically, the surface layer of the enamel remains intact while demineralization continues in the subsurface, but if no treatment is applied, it will eventually develop into complete cavitation.⁴ Saliva, which has an almost neutral pH, has a natural buffering capacity. In the early stage of demineralization, saliva reverses this process with calcium ions, phosphate ions, buffering agents, fluoride, and other substances.⁵

Various methods and agents are available for remineralization of the early enamel

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caries lesions. Topical application of mineral-rich concentrates can be used for remineralization of such initial lesions.⁶ Some of these agents can be referred to as traditional remineralization agents since they are known and used for a long time. Fluoride-based dental products and materials are frequently used to increase the resistance of the teeth to caries, and the remineralization effects of fluoride-based materials of different densities and shapes have been revealed in many studies.^{5,7} In addition to fluoride-based materials, those containing calcium, phosphate, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), and casein phosphopeptide amorphous calcium fluoride phosphate (CPP-ACFP) have been reported to be effective for remineralization.^{1,8,9}

Apart from these agents, new remineralization agents are also available today. These agents are generally produced and/or used in combination with fluoride. NovaMin, Xylitol + Hydroxyapatite, and ozone can be referred to as novel remineralization agents.

De- and re-mineralization of teeth can be detected by both invasive and non-invasive methods. A WSL cannot be detected visually until it has advanced 200-300 μm into the enamel.¹⁰ Non-invasive diagnostic methods include quantitative light-induced fluorescence (QLF), electrical resistance decay monitoring devices, fibre-optic transillumination, optical coherence tomography, laser fluorescence (DIAGNOdent), and scanning electron microscopy (SEM).¹¹ This study was performed to evaluate the efficacy of six different remineralization agents in vitro with the DIAGNOdent laser fluorescence method.

Methods

This in-vitro study was carried out at Dicle University Faculty of Dentistry in Diyarbakir, Turkey. Approval was received for this study from the local ethics committee (reference number: 2013; 1-7).

Preparation of enamel samples: To obtain standard affected enamel surfaces,

44 extracted, formerly impacted wisdom teeth lacking caries were used. The roots of the teeth were separated using an Isomet water-cooled microtome with a double-sided diamond thin blade, and the crown parts of the samples were separated along the occlusocervical and mesiodistal planes. On the buccal and lingual surfaces of the 88 samples obtained (11 samples for each group), labels measuring 3×3 mm were adhered to prepare 3×3 -mm² windows approximately 1-2 mm above the cemento-enamel junction, and the tooth surfaces outside the label were coated with nail polish. Finally, the enamel samples were randomly divided into eight groups, consisting of two control and six experimental groups as follows:

Group 1. Positive (+) control group: Demineralization cycle only

Group 2. Negative (-) control group: Intact tooth with nothing applied

Group 3. Fluoride gel group: Application of Topex® APF gel

Group 4. CPP-ACP cream group: Application of GC Tooth Mousse containing 10% CPP-ACP

Group 5. CPP-ACP + F cream group: Application of MI Paste Plus containing 0.2% (w/w) (900 ppm) NaF in addition to 10% CPP-ACP as casein phosphopeptide amorphous calcium phosphate, forming a CPP-ACFP cream

Group 6. NovaMin-Fluoride-containing toothpaste group: Application of Sensodyne Repair and Protection toothpaste, a toothpaste containing NovaMin and fluoride

Group 7. Xylitol-Hydroxyapatite-Fluoride cream group: Application of Remin Pro, a cream containing xylitol, hydroxyapatite (HA), and fluoride

Group 8. Ozone-Fluoride group: Application of PrOzone ozone generator and Topex® APF gel

Artificial initial enamel caries creation: Initial enamel caries were created in each of the groups except Group 2 by applying a de-/re-mineralization cycle (pH cycle), which was similar to clinical conditions. The pH cycle model was used for the evaluation of

materials contributing to the mechanism of caries generation under laboratory conditions by mimicking these phases. The pH cycle used in the present study was administered as a series of 24-hour periods for 9 days.

Preparation of de-/re-mineralization cycle: First, the samples were stored for 6 hours in the demineralization solution which included 1.5 mM CaCl₂, 0.9 mM KH₂PO₄, and 50 mM acetic acid with a pH of 4.3 at 37 °C. The samples were then removed from this solution and washed with distilled water. The samples were next stored in the remineralization solution which consisted of 1.5 mM CaCl₂, 5 mM KH₂PO₄, and 100 mM acetic acid with a pH of 7.0 at 37 °C for 17.5 hours. The samples were then removed from this solution, washed with distilled water, and placed back in the demineralization solution. This pH cycle was continued for 9 days, and the solutions were changed every 3 days to prevent any saturation.

Application of remineralization agents: The samples were divided into groups and artificial initial enamel caries were formed, except in the positive and negative control groups, Group 1 and Group 2, in which no remineralization agent was applied. In Group 3, the teeth were lightly dried according to the manufacturer's instructions, after which the APF gel was applied for 4 minutes.

CPP-ACP and CPP-ACP + F creams were applied to the teeth in Groups 4 and 5. Briefly, the teeth were lightly dried according to the manufacturers' instructions, and then the creams were applied for 3 minutes. In Group 6, toothpaste containing NovaMin and fluoride was applied for 2 minutes, mimicking the typical tooth brushing time. In Group 7, cream containing xylitol, HA, and fluoride was applied for 3 minutes after the tooth surfaces were lightly dried according to the manufacturer's instructions. In Group 8, PrOzone was used as an ozone generator while ozone gas was applied. Following ozone application, fluoride gel was applied to the teeth for 4 minutes.

All samples were then washed with distilled

water and incubated for 30 minutes at 37 °C in artificial saliva to simulate clinical conditions.

Laser fluorescence (DIAGNOdent) measurements: The laser fluorescence measurements were made using a DIAGNOdent (KaVo) device with a diode laser fluorescent beam with a wavelength of 655 nm and peak power of 1 mV. During the DIAGNOdent measurements, all samples were first calibrated with ceramic standard in accordance with the manufacturer's instructions. After calibration, the enamel surfaces were dried for 5 s with air. Next, the tip of the device was first touched to an area away from the surface to be measured to obtain a base value for that tooth. The tip of the device was then gently moved over the enamel surface and the maximum peak value was read from the screen on the front panel of the device. Measurements were repeated twice for each tooth, and the results were averaged and recorded. All the measurements were conducted by an experienced paediatric dentist.

Statistical analyses were performed with SPSS software (version 23, IBM Corporation, Armonk, NY, USA). To determine the sample size for the method used, a statistical power test (G*Power software) was performed. The minimum estimated sample size for each group was three samples, calculated considering 95% power and a significance level of 0.050 according to a previous study.¹² Descriptive statistics, as well as Wilcoxon and Kruskal-Wallis tests were employed to analyze the data. Bonferroni correction post hoc test was used for pairwise comparisons. In all analyses, $P < 0.050$ was taken to indicate statistical significance.

Results

The mean scores obtained after the measurements with DIAGNOdent were 3.82 and 6.91 in the negative-control group (Group 2) and positive-control group (Group 1), respectively. The mean scores of the experimental groups before and after remineralization are shown in table 1.

Table 1. Comparison of pre- and post-treatment DIAGNOdent scores

Experimental groups		Mean \pm SD	Median (Min-Max)	Test statistics	P
Control groups	Positive	6.9 \pm 2.1	7 (4-10)	Z= - 2.968	0.003*
	Negative	3.8 \pm 1.0	4 (2-5)		
F	Before	4.5 \pm 0.7	4 (4-6)	Z= - 3.051	0.002*
	After	3.5 \pm 0.8	4 (2-5)		
CPP-ACP	Before	4.3 \pm 0.8	4 (3-6)	Z= - 3.000	0.003*
	After	3.5 \pm 0.7	3 (3-5)		
CPP-ACP+F	Before	5.0 \pm 0.8	5 (4-6)	Z= - 3.127	0.002*
	After	3.2 \pm 0.6	3 (2-4)		
NovaMin + F	Before	4.5 \pm 1.2	4 (3-7)	Z= - 2.970	0.003*
	After	3.3 \pm 0.6	3 (2-4)		
Xylitol-HA + F	Before	4.9 \pm 0.8	5 (4-6)	Z= - 3.127	0.002*
	After	3.7 \pm 0.8	4 (3-5)		
Ozone + F	Before	5.2 \pm 1.2	5 (3-7)	Z= - 2.972	0.003*
	After	4.1 \pm 0.8	4 (3-5)		

SD: Standard deviation; F: Fluoride; CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP); Z: Wilcoxon test statistics, *P < 0.050

There were significant differences between the scores before and after the remineralization procedure in all experimental groups (Table 1).

Given the comparison of the mean differences in the pre- and post-treatment scores between the groups, the greatest difference was observed in the control groups. CPP-ACP + F provided significantly greater remineralization than other experimental groups (P < 0.050). This was followed by the NovaMin + F and xylitol + HA + F groups, which showed similar rates of remineralization, and then by the ozone + F, Fluoride, and CPP-ACP groups, respectively (Table 2).

Discussion

The results demonstrate two issues. First, statistically significant remineralization was achieved in all experimental groups. Second, the greatest difference between the mean scores measured with DIAGNOdent before

and after remineralization in the experimental groups was seen in the CPP-ACP + F group.

Different methods can be used to evaluate in-vitro de-/remineralization. Previous studies have used one or more tests, including scanning electron microscopy (SEM)/field emission scanning electron microscope (FE-SEM), DIAGNOdent, atomic force microscopy (AFM), and surface microhardness.^{5,13,14} Because DIAGNOdent has been reported to be a reliable non-invasive means of caries diagnosis,^{15,16} this method was used in the present study as well.

In this study, in which traditional and novel remineralization agents were compared, fluoride and CPPACP, which have long been used for remineralization and have been tested by researchers in many studies, have been classified as traditional remineralization agents. Moreover, the combined uses of these agents, which are reported to have synergistic effects, were included in this class.

Table 2. Intergroup comparison of mean differences in the experimental groups (n = 11)

Experimental groups	Mean difference \pm SD	Median(Min-Max)	Test statistics	P
F	1.00 \pm 0.45	1 (0-2) [#]	$\chi^2 = 22.11$	< 0.001
CPP-ACP	0.82 \pm 0.40	1 (0-1) [#]		
CPP-ACP + F	1.82 \pm 0.40	2 (1-2) [¥]		
NovaMin + F	1.18 \pm 0.75	1 (0-3) [#]		
Xylitol-HA + F	1.18 \pm 0.40	1 (1-2) ^{#¥}		
Ozone + F	1.09 \pm 0.54	1 (0-2) [#]		

SD: Standard deviation; F: Fluoride; CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP); ^{#¥}There is no difference between countries with the same symbol, χ^2 : Kruskal-Wallis test statistics, Post hoc test: Bonferroni correction for pairwise comparisons

NovaMin and xylitol-containing products, which have been released and popular in recent years, and applications that have recently been used for remineralization, such as ozone with fluoride, have been evaluated as novel remineralization agents.

In this study, in which traditional and novel remineralization agents were compared, fluoride and CPPACP, which have long been used for remineralization and have been tested by researchers in many studies, have been classified as traditional remineralization agents. Moreover, the combined uses of these agents, which are reported to have synergistic effects, were included in this class. NovaMin and xylitol-containing products, which have been released and popular in recent years, and applications that have recently been used for remineralization, such as ozone with fluoride, have been evaluated as novel remineralization agents.

Altenburger et al. examined the efficacy of CPP-ACP with DIAGNOdent and reported that the lesion size decreased significantly in the group treated with CPP-ACP.¹⁷ Uysal et al. compared a 5-minute CPP-ACP (Tooth Mousse) treatment group with a 5-minute 5% NaF (Fluoridine N5) treatment group, and reported that both caused similar increases in microhardness.¹⁸ In the present study, the results of the DIAGNOdent measurements indicated that Topex® APF gel produced significantly more remineralization compared to CPP-ACP ($P < 0.050$).

Fredrick et al. divided 45 teeth with occlusal WSLs detected by DIAGNOdent into three groups.¹² For 30 days, 10% CPP-ACP + 0.2% (w/w) NaF (Tooth Mousse Plus), 10% CPP-ACP (Tooth Mousse), and 0.5% (w/w) NaF were applied to the first group, second group, and third group, respectively. Measurements were obtained with DIAGNOdent, and the results showed similar remineralization potentials in Group 1 and Group 2, both higher than those in Group 3.

In the present study, the greatest difference between the average scores before and after remineralization was found in the

CPP-ACP + F group, and this difference was statistically significant ($P < 0.050$). Similar to the results of the present study, Elsayad et al., Karlinsey et al., and Patil et al. reported that the addition of fluoride to CPP-ACP had a synergistic effect on the remineralization of enamel.^{5,19,20}

Leila et al. reported that CPP-ACFP was more effective in achieving remineralization than Remin Pro.¹³ The DIAGNOdent results of the present study showed that the difference between the scores measured before and after the Remin Pro treatment was significant ($P < 0.050$), but the difference in the mean scores was lower than that observed with CPP-ACP + F.

Several studies have shown that ozone gas is effective for remineralization.^{16,21,22} In the current study, the difference between the measurements before and after ozone treatment was significant, and the difference between the measurements was higher in the ozone group in comparison to the APF and CPP-ACP groups, but lower in the other groups.

This study had some limitations, with the first being DIAGNOdent, as although it is known as a reliable tool for the detection of initial carious lesions, it should be supported by other methods. The results indicated by DIAGNOdent are not as precise as those demonstrated by SEM, which may have affected the accuracy of the present results. In the groups included in the class of novel remineralization agents, fluoride was available as a combined agent. The level of contribution of main content and fluoride to remineralization was exactly unknown.

Despite these limitations, according to the results obtained from this study, it can be claimed that the traditional remineralization agents are still effective compared with the novel agents.

Conclusion

The de-/re-mineralization solutions and pH cycle model used in this study were sufficient to create artificial WSLs in vitro.

The DIAGNOdent device can be used for caries detection in in vivo and practical conditions, providing rapid and repeatable measurements in a non-invasive manner.

Statistically significant remineralization was achieved in all experimental groups.

The greatest difference between the mean scores measured with DIAGNOdent before and after the remineralization in the experimental groups was seen in the CPP-ACP + F group.

The traditional remineralization agents are still effective compared with the novel agents.

Conflict of Interests

Authors have no conflict of interest.

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