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ORIGINAL RESEARCH

The effect of remineralizing agents on artificial caries lesions by laser Fluorescence

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Abstract

Background: The present study aimed to use a DIAGNOdetnt unit to compare the effects of casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF), 0.05% sodium fluoride (NaF) mouth rinse, and glass ionomer (GI) on enamel remineralization.

Materials and Methods: This in vitro study was performed on 50 intact human premolars in five groups: A (artificial saliva), B (CPP-ACPF), C (NAF), D (GI), and E(GI+NAF). All samples were assessed using a DIGNOdetnt unit before and after demineralization. The teeth in each group were treated (except for the control group) then pH cycled repeatedly for 30 days. After this, the remineralization evaluation was evaluated. Data gathered by DIAGNOdetnt were analyzed by One-way analysis of variance. The least significant difference (LSD) test was used for multiple comparisons (α=0.05).

Results: After the treatment, the mean DIAGNOdetnt values in groups B(3.6), C(4.7), D (5.1), and E(3.5) were significantly lower than that in group A(P<0.0001). LSD test showed that the mean DIAGNOdetnt value in groups B and E was lower than for groups C and D(P<0.001).

Conclusion: Although the repeated application of all the experimental materials was efficient, CPP-ACPF and GI+NAF had more prominent remineralization potential in comparison to NAF and GI alone.

Keywords: CPP-ACPF, DIAGNOdetnt, Glass ionomer, NAF rinse, Remineralization.

Introduction

The oral cavity is a place where demineralization and remineralization compete. Demineralization happens when the pH in the oral environment drops below 5.5, allowing calcium and phosphate ions to diffuse from the enamel surface (1). This usually appears as a white spot lesion, which is a common side effect of using fixed appliances for orthodontic treatment (2). Remineralization can occur when the pH rises, and there are enough calcium, phosphate, and fluoride ions to reenter the tooth structure. Minerals available in saliva and fluoride can have a synergistic effect to arrest or reverse demineralization (3).

Therefore, diagnostic tools for early caries detection can help reverse the process and reduce the need for operative treatment. The traditional methods of diagnosing caries include visual, tactile, and radiographic evaluation which are highly subjective and cannot be appropriate for the mentioned purposes (4,5). DIAGNOdetnt (KaVo, Liberace, Germany) is a non-invasive method that uses laser fluorescence technology to detect early demineralization. The tooth surface absorbs the red light; of wavelength 655 nm and emits fluorescence above 680 nm. The device detects the exhibited fluorescence and quantifies it as a value between 0-99(6). Higher digital readings on the display (also heard through an audio signal) indicate greater demineralization.

Studies show that fluoride is the most effective for remineralization, provided in various forms of rinse, gel, varnish, and dentifrice. (7-11).

The protective effects of milk and its products against dental caries are widely recognized due to the presence of casein, calcium, and phosphorus which are responsible for resistance to acid dissolution(1). Casein phosphopeptide-amorphous calcium phosphate(CPP-ACP) was introduced by Reynolds EC as a product drive from milk casein(12). Considering the benefits of F and CPP-ACP, a new product called MI paste

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Plus (GC Corp., Tokyo, Japan) has been developed by combining 900 ppm F and CPP-ACP (13). Some studies have shown that remineralization by CPP-ACPF is more efficient than using CPP-ACP alone (14).

Dentists who specialize in treating children must address lesions that cannot be cleaned daily, but using too many methods can lead to swallowing problems (2). A newer approach involves incorporating fluoride (F) into dental materials like glass ionomer cement (GIC) (15). This means that using restorative materials containing F can be beneficial because they release F into the mouth (16).

This study aimed to evaluate the in vitro effects of CPP-ACPF, 0.05% sodium fluoride(NaF) mouth rinse glass ionomer(GI), and the combination of 0.05% NaF mouth rinse and GI (Naf +GI) on remineralization of artificial caries lesions using a DIAGNOdetnt pen.

Materials and Methods

Fifty premolars were carefully collected and preserved for orthodontic purposes using a 0.2% thymol solution from Applichem GmbH, Ottweg 4, D-64291 Darmstadt, Germany. Healthy teeth were enrolled in this study, and any teeth exhibiting caries or enamel defects, such as spot lesions, enamel hypoplasia, cracks, or gross irregularities, were excluded. Soft tissue and calculus were removed (17) then, a white 4x4 mm2 stick-on paper was placed on the middle third of the buccal surface. Acid-resistant nail varnish was applied to all crown surfaces to isolate the study area. (1).

Baseline DIAGNOdetnt measurement

The wireless DIAGNOdent pen (Ka Vo in Liberace, Germany) was used to measure the Baseline DIAGNOdent reading of all samples, following the manufacturer's instructions. The DIAGNOdent unit had two types of probe tips: one for pits and fissures (type A) and another for smooth surfaces (type B). For this study, we used probe type B. Baseline DIAGNOdent values were recorded for all samples, and teeth with readings of 0-70 on the digital display (according to the manufacturer's catalog) were selected (Table 1).

Table 1. DIAGNOdetnt interpretation

Moment Values	Inference
0-7	Healthy tooth substance
8-15	Beginning demineralization
>16	String demineralization

Experimental and control groups

The 50 samples were immersed individually into separate plastic containers numbered from 1 to 50. They were divided into five groups as follows:

group A (Control) artificial saliva (3.9 mmol/l Na3PO4, 429 mmol/L Nacl2, 17.98 mmol/L KCL, 1.1 mmol/L Cacl2, 0.08 mmol/L Mgcl2, 0.5 mmol/L H2SO4, 3.27 mmol/L NaHCO3, pH = 7.2) (18).

Group B: CPP-ACPF (MI Paste Plus)

Group C: NAF mouth rinse (Epimax, Emad Pharmaceutical Co., Iran)

Group D: High Glass ionomer (EQUIA TM File, GC Corp., Tokyo, Japan)

Group E: High viscosity Glass ionomer (EQUIA TM Fil, GC Corp., Tokyo, Japan) Plus 0.05% NaF mouth rinse (Epimax, Emad pharmaceutical Co., Iran)

Demineralization Procedure

The teeth were immersed in 20 ml demineralizing solution (2.2 mmol/L Cacl2, 2.2 mmol/L NaH2PO4, 0.05 mmol/L lactic acid, pH=4.5) for 72 hours. This procedure was performed in an incubator at 37°C (18). After the mineralization, the samples were rinsed with distilled water, dried, and placed back in their clean containers. After induction of enamel lesions, all samples with DIAGNOdetnt values between 8 and 15, indicating the presence of a subsurface space lesion (according to the manufacturer catalog), were taken for further evaluation.

PH cycling regimen

For one month, the pH cycling regimen alternated between demineralization (three hours) and remineralization (21 hours). The samples underwent demineralization phases followed by treatment with remineralization agents and then placed in artificial saliva to simulate the natural oral cycle. Group A samples were exposed to the pH cycling regimen without any treatment, while group B samples were rubbed with 1g CPP-ACPF cream by an applicator brush and left undisturbed for a minimum of three minutes daily. Each sample in group C was immersed in NaF mouth rinse for one minute daily. Group D samples were exposed to GI (glass as a device with a diameter of 4mm and about 2 mm thick on the buccal surface using conditioner), and group E samples were exposed to GI+NaF (glass as a device with a diameter of 4mm and about 2 mm thick on the buccal surface using conditioner and immersed in NaF mouth rinse for one minute every day). After the remineralization procedure, all samples were assessed using DIAGNOdetnt, and the new values were recorded. The DIAGNOdetnt reading values of groups D and E were assessed surrounding the device.

Statistical analyses

To ensure accuracy, we first assessed and confirmed the normality of the research data using the Kolmogorov-Smirnov test. We then utilized a one-way analysis of variance (ANOVA) to compare changes in DIAGNOdent measurements during the demineralization procedure. Multiple comparisons were conducted using the least significant difference (LSD) test. Analysis of data was conducted using SPSS 26.($\alpha<0.05$)

Results

According to the results of a one-way ANOVA, there were no significant differences in the average baseline

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DIAGNOdent values (p=0.903) among the five groups before demineralization. Although the mean DIAGNOdent values for all groups increased after demineralization, there was no significant difference between the five groups (p = 0.986). However, significant differences were seen in the mean DIAGNOdent values after treatment (p < 0.001) (Table 2).

Table 2. Comparison of the mean DIAGNOdetnt value

Groups	Before demineralization	After demineralization	After treatment
	Mean± SD*	Mean± SD	Mean± SD
Control	5.5±1.1	9.2±1.5	7.8±1.4
CPP-ACPF	5.8±0.6	9.5±2.5	3.6±0.5
NaF rinse	5.4±0.7	9.4±2.1	4.7±1
GI	5.7±1.5	9.7±1.8	5.1±0.6
GI+ NaF	5.5±1	9.4±1.7	3.5±0.5
P value	0.903	0.986	<0.001

* standard deviation

Various comparisons revealed that the average DIAGNOdent values of groups B, C, D, and E were notably lower than that of the control group, i.e., group A. Moreover, the mean DIAGNOdent values of groups B and E were significantly lower than those of groups C and D. However, no significant differences were observed between groups B and E or groups C and D, as shown in (Table 3).

Table 3. Pairwise comparison of means after treatment

Groups	Control	CPP-ACPF	NaF	GI	GI+ NaF
Control	-	S	S	S	S
CPP-ACPF	S	-	N	S	N
NaF rinse	S	N	-	N	N
GI	S	S	N	-	S
GI+ NaF	S	N	N	S	-

S: p< 0.05, N: P>0.05

Discussion

Modifying caries depends on the application of both preventive and early detection strategies. Thus, using non-invasive caries detection methods, such as quantitative light fluorescence (QLF), fibre-optic transillumination, optical coherence tomography, and laser fluorescence (DIAGNOdent), is of importance.

This research employed the DIAGNOdent technique to analyze enamel surface changes before and after demineralization and after remineralization treatment. Previous studies have extensively examined this method for detecting cavities on both occlusal and smooth surfaces (21, 24).

Using 0.05% NaF mouth rinse significantly increased remineralization compared to the control group (p< 0.0001). various studies have similarly suggested the efficacy of 0.05% NaF mouth rinse

in enamel lesion remineralization (26-28). Although we applied the mouthwash once daily as recommended by the manufacturer: Songsiripradubboon et al. (27) concluded that twice daily use of 0.05% NaF mouth rinse could cause the remineralization of incipient caries.

MI Paste Plus, which contains CPP-ACP and 0.2% F, significantly increased remineralization compared to the control group as revealed by researchers, such as Srinivasan et al (29). Jayarajan et al (1) investigate the efficacy of CPP-ACPF for remineralizing the enamel surface. It was found that CPP-ACP induced a higher level of remineralization than CPP-ACP alone. Rawal et al (13) used CPP-ACP once daily in their protocol and evaluated its ability to prevent enamel demineralization adjacent to orthodontic braces in vitro. They concluded that regular daily application of CPP-ACPF in orthodontic patients would provide maximum protection against enamel demineralization by reducing lesion formation and promoting simultaneous remineralization.

Based on our findings, CPP-ACP was significantly more effective than NaF mouth rinse in increasing remineralization. This highlights the synergistic effect of F combined with CPP-ACP and F. They have reported CPP-ACP in inducing remineralization. Numerous studies (e.g., Reynolds et al (30) and Elsayad (31)) have confirmed greater enamel remineralization because of using a combination of CPP-ACP and F rather than the application of CPP-ACP or F alone. Conversely, some in vitro studies (e.g., Kumar et al (32); Lata et al (18)) have failed to show the synergic effect of CPP-ACP and F. They have reported CPP-ACP to be less efficient than F and the combination not to have more remineralization potential than F (18, 32). The possible reasons for much inconsistency might be the inability of F ions and ACP-CPP to penetrate the surface enamel area, failure to exactly mimic the in vivo conditions occurring in the mouth through the in vitro set-up, and too short duration of the experimental set-up (seven days).

Group D samples showed a more noticeable increase in enamel remineralization in comparison to the control group. Previous studies have established the beneficial effect of GI on enamel remineralization. (33-36). However, we did not detect any significant differences between the effect of GI and NaF (p= 0.305). Zhou et al (37) compared the effects of five materials on remineralization of artificial enamel lesions and concluded that GIC-based dental materials can promote remineralization better than NaF-based dental materials. Vojinović et al (2) discovered that early carious lesions were better protected with GIC. Hatibovic-Kofman (34), Mueller et al (33), and Trairatvoraku et al (38) proved GIC to be more efficient than F varnishes and F-releasing sealants. Marinelli et al. (39) studied enamel remineralization after using F rinse, fluoridated toothpaste, and F-releasing restorative materials. They discovered that F rinse had a better effect on caries remineralisation compared to other methods. This might be because F rinse was applied twice a day, whereas, in the current research it was applied once a day.

According to our research, the combination of GI and Naf was more effective than using GI and F alone.

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Kupietzky et al. (40) found that fluoride-treated GI released more fluoride than untreated GI.

Freedman et al. (42) studied fluoride release from three distinct restorative GI-based materials. The findings indicated that an increase in daily fluoride exposure resulted in an enhanced release of fluoride for all three restorative materials. The study confirmed that GI and fluoride have a synergistic effect and that GI materials can absorb and release significant amounts of fluoride. This can help in enhancing the preventive effects against tooth decay. (40, 41). Nevertheless, Rao and Sudha (43) rejected the synergistic effect of GI and local F. They measured the released ion of 1000ppm fluoridated dentifrice on self-cured GI by digital ion analyzer at 1, 2, 7, 15 and 30 days and concluded that despite that high F release, when Fuji VII was exposed to a 1000 ppm fluoridated dentifrice, it distilled water (which is not identical to the enamel cycle in the mouth). In addition, they analyzed the ions in distilled water rather than tooth remineralization.

According to (Table 1), the mean DIGNOdent values in the five groups were not significantly different either before or after demineralization (P= 0.903 and 0.986, respectively). Therefore, the five groups can be more reliably compared after remineralization.

Although short PH cycling periods have been used in most recent research, e.g., studies performed by Oliveira et al. (11) (10 days), Jayarajan et al (1) (one week), Pai et al (44) (two weeks) and Uysal (17) (three weeks), we continued PH cycling for one month. Since the CPP-ACPF manufacturer recommended its application for one month, our findings are more dependable than others.

Meanwhile, despite the use of a single method (DIGNOdent) for remineralization assessment in the present study, the application of two or more methods, including scanning electron microscopy (SEM) and QLF, would have been more appropriate

Most recent studies have applied GI for more than one month and examined the effect of slow F release in longer periods (2, 39). However, we limited the exposure to one month. An extended study period might lead to more reliable results.

Conclusion

CPP-ACPF, NaF, GI, and GI+NaF showed promise in decreasing enamel mineral loss. ACC-CPPF and GI+NaF were particularly effective in preventing caries. This indicates that the addition of F may have a synergistic effect on reducing demineralization and promoting remineralization.

Conflict of Interests: None

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