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Casein Phosphopeptides-Amorphous Calcium Phosphate's Effect on Microhardness of Teeth Treated With 5% Sodium Fluoride

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Abstract

Background: Dental caries is a common disease among humans, and its significant impact underscores the need to promote the mineralization of early enamel lesions. Numerous materials have been investigated to enhance the mineralization of teeth. Casein phosphopeptides-amorphous calcium phosphate (CPP-ACP), a milk protein known for its effectiveness in mineralization of tooth.

Aim of study: To assess CPP-ACP's effect on the enamel microhardness of teeth treated with 5% sodium fluoride.

Materials and Method: Forty extracted sound upper first premolars were utilized in the study. The teeth underwent a pH cycle, after which, 20 teeth were treated with a 5% sodium fluoride solution (NaF), while the remaining 20 to deionized water. All teeth were placed in artificial saliva for 24 hours and subsequently treated with 10% CPP-ACP for seven days. Enamel microhardness was evaluated before and after the artificial formation of caries-like lesions, following the application of 5% sodium fluoride and deionized water, and after the application of CPP-ACP on the previously treated groups.

Results: It was found that 5% NaF significantly elevated the microhardness of the enamel after demineralization (P=0.000), the mean microhardness value post-demineralization was (119.11), while post-remineralization was (195.910) and following the application of CPP-ACP it became (226.100) which was also significant (P=0.025).

Conclusion: CPP-ACP is an effective material for mineralizing early carious lesions alone, but it performs its best effect when combined with NaF.

Keywords: CPP-ACP, caries-like lesions, microhardness, NaF, remineralization.

Introduction

The contemporary caries management approach emphasizes identifying and treating early-stage carious lesions and important risk factors that lead to caries development. This approach is known as minimum intervention dentistry (Warreth, 2023). This philosophy emphasizes nonoperative therapies with the goals of minimizing invasion and maximizing tooth structural preservation.

Many preventive strategies target the remineralization of enamel to stop or reverse the early stages of tooth decay before it worsens. For this purpose, two commonly used substances are NaF and CPP-ACP. NaF assists in the creation of fluorapatite, a substance that exhibits greater resistance to acid attack (Jabin *et al.*, 2021).

Sodium fluoride has a long history of use in preventing dental cavities. It enhances the process of remineralization of enamel by integrating it into the crystal structure of the enamel, hence increasing its resistance to demineralization (Featherstone, 2000). Many researchers have repeated demonstrated that fluoride treatments can effectively decrease the occurrence of tooth decay and improve the process of restoring minerals to damaged enamel and dentin that has been damaged (Al-Asmar, 2024, Piszko *et al*., 2024, Alani and Qasim, 2014).

CPP-ACP is recognized for its capacity to enhance the concentration of calcium and phosphate ions inside the oral cavity, therefore facilitating the process of enamel remineralization. Evidence from several research has demonstrated the effectiveness of CPP-ACP in promoting the process of enamel remineralization (Elgezy *et al*., 2024, Akküç *et al.*, 2023, Cardoso-Martins *et al*., 2022). Its capacity to bind to calcium and phosphate ions creates a reservoir that gradually releases them to the tooth surface. Which, in turn, aids in the process of remineralization (Yengopal and Mickenautsch, 2009).

The aim of the study is to investigate the effect of CPP-ACP preforms on teeth that have been previously treated with NaF.

Material and methods

The current study was conducted at the University of Technology, Metallurgy and Manufacturing Engineering Department, and the Nanotechnology Department. And at University of Baghdad, Geology Science Department. After being authorized by the scientific committee of the College of Dentistry, Mustansiriyah University approval number (MUPRV005).

Forty sound, unaffected teeth were utilized in this in vitro study and examined using X6 dental loops (Univet, Italy) (Boutsiouki *et al.*, 2019). The teeth were cleaned with ultrasonic cleaner and polished with fluoride-free surface polish (Yahya and Rawi, 2020). The buccal surface was coated with nail varnish (Deborah Milano, Italy) except for a 3x3 mm window at the center of the buccal surface (Valizadeh *et al.*, 2020).

Caries like lesion formation

The teeth were subjected to the Feather Stone pHcycle (Featherstone J., 1986). By alternately applying them in demineralized/remineralized solutions as follows: 6 hours at pH 4.3 in demineralizing solution (consisting of 1.0 M/L calcium chloride, 0.075 M/L acetic acid, 2.0 m M/L phosphate chloride) and 17 hours at pH 7 in remineralizing solution (consist of 0.9 m M/L potassium phosphate,150 m M/L potassium chloride,1.5 m M/L calcium nitrate) respectively. In between the solution, the teeth were rinsed for 60 seconds each with deionized water (Anni *et al.*, 2010).

5% sodium fluoride preparation

A 5% sodium fluoride solution was prepared by dissolving 0.5 g of the powder (Thomas Baker Chemicals, India) in 10 ml of deionized water in a volumetric flask (Swarup and Rao, 2012).

Study groups and remineralizing agents' application

Forty teeth were used in this study. They underwent a 10-day pH cycle to form caries-like lesions and then a two-step application. The first step involved dividing the teeth into two groups as follows:

5 % NaF group: 2 drops of sodium fluoride solution were dispensed on the teeth with a micro brush for 2 minutes.

Control group: 2 drops of deionized water were dispensed on the teeth with a micro brush

Then the samples were rinsed with deionized water and immersed in artificial saliva for 24 hours (Ata and S.M., 2019).

The second step, entails applying a 10% concentration of CPP-ACP (GC mousse, Tokyo) for exactly three minutes(Irmaleny *et al.*, 2023) on all groups, then rinsed once more with deionized water and placed in artificial saliva. That step was repeated twice a day for 7 days (Bhat *et al.,* 2022).

Surface Microhardness Assessment:

The microhardness measurements were assessed using a Vickers machine. Four measurements were carried out on the samples: baseline, after demineralization, after 1st step of remineralization (application of 5% NaF and deionized water), and a final one after application of CPP-ACP. The measurements were undertaken by applying a load of 300 grams for 15 seconds (machine instruction). Vickers Microhardness test has been used in many previous studies (Al-Nuaimi and Gasgoos, 2023, Jamwal *et al.*, 2022, Mansoor and Hashim, 2021).

Statistical analysis

Using SPSS for Windows (Statistical Package for the Social Sciences), we assessed the normality of the data was carried out through Shapiro–Wilk analysis, the data were analyzed using descriptive statistics analysis of variance (ANOVA), and the least significant difference (LSD) test.

The results

Descriptive analysis is presented in Tables 1 and 2. The analysis of variance (ANOVA) and the least significant difference (LSD) analysis of microhardness values are shown in Tables 3 and 4, showing the minimum, maximum, mean, and standard deviation of values included in descriptive analysis, along with the degree of freedom and significance of the ANOVA test. The LSD analysis indicates the mean differences and significance of each stage of each group. The normality of the data was checked with the use of the Shapiro-Wilk analysis

The result showed a P-value (P=0.000), indicating a statistically significant change between the baseline and demineralization phases in all groups. This difference is attributed to a considerable drop in microhardness values after demineralization compared to the baseline phase. The examination of the first stage of remineralization revealed a substantial rise in microhardness values in the NaF groups, with a (P=.000), as shown in Table 3. In contrast, the control group showed no significant change (P=.483) between the demineralization stage and deionizes water stage, as indicated in Table 4.

After the second stage of remineralization, the microhardness values were significantly different (P=.025) when comparing 1st stage remineralization of the NaF group with the CPP-ACP stage, as shown in Table 3. On the other hand, the p-value was highly significant (P=0.000) between the first stage of remineralization of the control group and the CPP-ACP stage, as shown in Table 4.

Discussion

Recently, there has been a growing awareness of dental caries and its management, particularly regarding non-invasive methods for addressing early carious lesions. The goals of these methods are to preserve tooth structure and prevent the progression of lesions (Tassery *et al.*, 2024, Desai *et al.*, 2021).

The present investigation aimed to determine the effect of CPP-ACP on teeth previously treated with a 5% sodium fluoride solution and to compare it with teeth treated with CPP-ACP alone.

In the present investigation, the mean baseline microhardness value for the samples was (327±53) kgf/mm^2, corresponding with the normal enamel microhardness range (Aldeen, 2022, Ahmadi Zenouz *et al.*, 2015).

Based on our findings, the microhardness values after the pH cycle showed a significant drop that was statistically significant (P<.05) in both the NaF and deionized water groups. This aligns with the results of other studies (Anni *et al.*, 2010, Al-sayyab, 2000) which indicate the effectiveness of the pH cycle and, the subsequent formation of caries-like lesions.

Sodium fluoride was the first fluoride product used in dentistry. It has a long history of use and is highly regarded for its efficacy; many researchers have utilized it in their studies to assess the effectiveness of new materials in the remineralization of early caries lesions (Deulkar *et al*., 2023, Farhad *et al*., 2021).

After applying 5% sodium fluoride to one of the groups, it showed a statistical increase in microhardness values ($P<0.05$), compared to that after demineralization, as seen in other studies investigating sodium fluoride.(Jahanimoghadam *et al.*, 2024, Baik *et al.*, 2021) Indicating the transportation of the minerals to the demineralized areas.

Microhardness values of the deionized water group showed a nonstatistical significance ($P >0.05$) when compared to those after demineralization. Conforming the non-mineralizing capacity of deionized water.

CPP-ACP induces remineralization by incorporating the CPP component into the enamel. This integration facilitates the delivery of calcium and phosphate ions to the enamel rods, promoting the reconstruction of apatite crystals (Nadar *et al*., 2022, Li *et al*., 2014).

The application of CPP-ACP led to an increase in enamel microhardness in both groups. The NaF group showed a significant difference ($P<0.05$) when compared to the first remineralization stage and ($P<0.001$) for the deionized water group when comparing the CPP-ACP stage to the deionized water stage.

The mean of microhardness values following the application of CPP-ACP on the deionized water group was (169.92) kgf/mm^2, with statistical significance ($P<0.001$), which is similar to those of Tantbirojn *et al*., (2008) who demonstrated that demineralized enamel underwent substantial hardening following treatment with 10% CPP-ACP (Tantbirojn *et al*., 2008). A systematic review conducted by Ekambaram *et al*. showed that products containing CPP-ACP have a superior enamel remineralization effect compared to other treatments based on calcium and phosphate (Ekambaram, 2017). Many previous investigations have revealed that CPP-ACP increases teeth hardness after demineralization (Nishna *et al.,* 2023, Bhat *et al.,* 2022, Reise *et al*., 2021, Thierens *et al*., 2019).

The mean microhardness values of teeth treated with CPP-ACP following NaF was (226.100) kgf/mm^2, with statistical significance ($P<0.05$), which reflects the synergic effect CPP-ACP had on NaF as seen in other studies (Attiguppe *et al.,* 2019, Shen *et al*., 2016).

Also, these findings are accords with Tao *et al*., (2018); Duraisamy *et al*.,(2015) which conformed the additive effect of CPP-ACP when combined with NaF (*Tao et al*., 2018, Duraisamy *et al*., 2015).

Meanwhile, Shahmoradi *et al*.'s study examined several fluoride varnishes and their effects on mineralization. They concluded that adding calcium and phosphate compositions had no discernible impact on the effectiveness of the fluoride varnishes' effectiveness (Shahmoradi *et al*., 2017). This contrasts with the current study due to the notable increase in microhardness in all groups after application of CPP-ACP.

The limitation of the study can be summarized by the short duration of CPP-

ACP application, lack of plaque biofilm and dietary habits that can affect the results when preformed on individuals. It should note that CPP-ACP plus NaF has been widely assessed and compared in literature, however, in this study different protocol was considered during the application where the above two materials were applied in different time points and showed promising results.

Conclusion

Sodium fluoride remains a fundamental component in the treatment of early dental caries. Based on its established mechanisms of action, effectiveness in clinical trials, and practical applications demonstrate its ability to suppress bacterial metabolism and stimulate remineralization.

On the other hand, CPP-ACP is an important therapeutic material for treating early dental caries lesions. Its ability to stabilize calcium and phosphate ions and promote remineralization makes it a promising product for the treatment of dental caries. It presents a possible substitute or supplement to conventional fluoride treatments, enhancing the fluoride effect on teeth. However, further investigation is required to comprehensively discern its enduring advantages, cost-efficiency, and optimal use in different therapeutic settings.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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Table1: Descriptive analysi s of microhardness values (kgf/mm2) of NaF group before and after CPP-ACP applica tion.

Stage	Min	max	mean	Std. deviation
Base	295.0	385.0	327.795	22.9774
Demineralization	54.6	193.6	119.115	36.9409
NaF	74.0	302.0	195.910	51.8414
CPP-ACP	115.3	297.0	226.100	48.9000

Table 2: Descriptive analysis of microhard ness valuesb (kgf/mm2) of the control (deionized water) group before and after CPP-ACP application.

Stage	Min	Max	Mean	Std. deviation
Base	281.6	378.6	327.96	27.203
Demineralization	55	190.1	113.19	36.454
Deionized	58	194	121.15	36.352
CPP-ACP	98	232	169.92	40.294

Table 3: ANOVA test and LSD analysis of the microhardness values of the NaF group before and after the CPP-ACP application.

Type / Stage	ANOVA			LSD		
	F -value	P -value	Degree of freedom	Pairwise comparisons	Mean difference	P -value
Baseline(1)	88.291	<.001	3	1vs 2	208.680	.000
Demineralization (2)				1vs 3	131.885	.000
NaF(3)				1vs 4	97.385	.000

CPP-ACP (4)				2vs 3	76.7950	.000
				2vs4	111.295	.000
				3 vs 4	34.500	.025
Statistical significant value (P<0.05)						

Table 4: ANOVA test and LSD analysis of the control (deionized water) group before and after CPP-ACP application.

Type / Stage	ANOVA			LSD		
	F -value	P -value	Degree of freedom	Pairwise comparisons	Mean difference	P -value
Baseline (1)	58.921	<.001	3	1vs 2	214.760	.000
Demineralization (2)				1vs 3	206.805	.000
Control(3)				1vs 4	158.040	.000
CPP-ACP (4)				2vs 3	7.955	.483
				2vs4	56.720	.000
				3 vs 4	48.765	.000
Statistical significant value (P<0.05)						