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Comparison of CPP-ACP, Fluoride Varnish and Gel effects on enamel microhardness of permanent teeth: *In-Vitro*

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Abstract

Background
Dental caries occurs due to the imbalance in the course of demineralization-remineralization in favor of demineralization. With remineralization, some signs of halted progression could be observed in the primary stages. The aim of this study is to in-vitro investigation of the effect of Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP), fluoride varnish, and fluoride gel on the microhardness of the enamel of permanent teeth.

Materials and Methods: In this in vitro study, 344 specimens prepared from human premolars were randomly divided into four groups (n=86 specimens in each group). After preparing the specimens, the initial enamel value was first evaluated by Vickers microhardness technique. The specimens were immersed in a demineralizing solution and then treated with remineralizing compounds (I. Control, II. Fluoride varnish, III. Fluoride gel and IV. CPP-ACP). Microhardness values were re-measured at the end of demineralization and remineralization stages. The data were analyzed using SPSS software (version 16.0).

Results: The results of the present study indicated a statistically significant difference in all groups in terms of initial enamel microhardness (0.288 ± 42.77), remineralization (0.213 ± 26.89), and demineralization values (0.167 ± 63.87) (p <0.001). Besides, there was a statistically significant difference in different groups in terms of remineralization values (p<0.001). In other words, the mean microhardness was 241.21 ±15.60 in Group III, 221.12 ± 75.69 in Group II, 211.16 ± 43.04 in Group IV, and 178.21 ± 63.9 in group- I.

Conclusion
All remineralizing compounds resulted in enhanced microhardness of the enamel. Nevertheless, the fluoride-containing products showed greater potential in improving the level of microhardness and strength of the teeth compared to the compounds containing CPP-ACP.

Key Words: CPP-ACP, Fluoride, Microhardness, Tooth enamel, Remineralization.

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1- INTRODUCTION

In spite of the descending trend of dental caries among children worldwide, this complication is still considered one of the most common diseases of this period. Dental caries causes pain and discomfort, while also affecting the quality of life (1). Accordingly, preventive measures should be incorporated in the treatment plan of patients in order to prevent this disease (2). Dental caries is a disease occurring in response to the imbalance between demineralization-remineralization in favor of demineralization at dental surfaces (3). When it occurs repeatedly over time, it leads to loss of a major part of the mineral tissue of the teeth or demineralization and then formation of carious lesions (4).

The tooth enamel is the most mineral tissue in the body, whose weight is composed of 96% minerals and 4% organic materials plus water (5). Evidently, loss of a major part of the mineral tissue of the teeth or demineralization followed by reduced hardness of the enamel play a key role in dental caries. This is because the dental tissue becomes exposed to the environmental factors of the oral cavity with the minimum resistance and defense coverage (6). In the past, clinicians believed that in order to manage dental caries, only an invasive and immediate approach such as removing the caries and then placing restoration materials can be used in order to prevent the progression of this disease completely. However, evidence collected throughout recent years has resulted in changes in this attitude (7).

Today, it is believed that aggressive treatment alone cannot halt the progression of dental caries, and other points should also be considered (8). Possibly, in the first glance, dental caries seems irreversible, because it leads to degradation of the healthy dental tissue. However, it is also possible that in the very early stages, with remineralization, some signs of halted progression of dental caries could also be observed (7, 9-12). Extensive evidence exists on the importance of fluoride as a mineral to prevent demineralization and begin remineralization (13-15). By attaching to the components of the tooth enamel, fluoride prevents the onset of lesion, slows down its progression, and can even stop the lesion development (16-18). Since the mechanism of fluoride is more effective across the dental plaque surface, numerous topical methods have been devised in order to deliver this material to the dental tissue. Examples of materials include toothpaste, mouthwash, varnish, gel, etc. (7). Fluoride varnish was produced in the late 1960s in order to improve the effect of anti-caries properties through increasing the contact time between the enamel and fluoride. Fluoride varnish has been classified as one of the most logical methods of fluoride transfer because of its ease of use and tolerability, especially for particular groups including infants, autistic patients, and patients with mental or physical disabilities (19-22).

Nevertheless, one of the disadvantages of fluoride varnish is the effect it leaves on the appearance of teeth. The yellow layer of varnish remains for some hours on teeth until it is removed by toothbrush. In addition, temporary discoloration after applying varnish on the teeth can itself create an unpleasant feeling in the patient. Further, many patients may be dissatisfied with the presence of the thin varnish layer on their teeth, or the unpleasant taste of this substance may be annoying for some patients (20). One of the solutions to locally transfer fluoride is to apply various types of liquid, gel, and foam. Since fluoride foam and gel are prepared through tray, the contact of all teeth simultaneously can be possible (23). Meanwhile, use of fluoride in gel form is important because of relatively high fluoride concentration. The risks of exposure to high doses of this type of fluoride can lead to acute toxicity.

Children undergoing treatment with fluoride gel have complained from nausea, vomiting, headache, and abdominal pain. Accordingly, because of the possible risks, use of gel in infants is not generally recommended (24). In addition, remineralizing agents apart from fluoride derivatives have recently been discovered which can significantly help clinicians in controlling dental caries. One of these agents, which has recently achieved popularity, is a substance containing active components of amorphous calcium phosphate plus casein phosphopeptide (CPP-ACP) (25). CPP is a protein obtained from milk which can bind to phosphate and calcium ions. CPP-ACP compound inside the oral cavity binds to dental plaques, hydroxyapatite, as well as soft tissue, thereby providing the calcium and phosphate required for remineralization in the saliva and dental plaque (26).

In the long run, the superior regionalization properties of products containing CPP-ACP have been reported in comparison to materials such as sodium fluoride varnish or Nano-hydroxyapatite (27). Although, according to some reports, CPP-ACP alone cannot be considered one of the best therapeutic options, combination of this material with fluoride can enhance the therapeutic outcomes (28, 29). Meanwhile, CPP-ACP compound according to some other researchers has no advantage over applying fluoride alone in preventing dental caries (11, 30).

Generally, since swallowing CPP-ACP is harmless and is also has a more favorable taste compared to other remineralizing materials, if it is able to establish better remineralization compared to varnish and gel, it seems to be a suitable, desirable, and safe alternative to current derivatives of fluoride-containing materials (31, 32). Considering the importance of understanding a safe and simple technique to prevent dental caries especially during childhood and regarding the limited studies performed on the influence of CPP-ACP as a new material, the aim of this study has been to in-vitro investigation the effect of CPP-ACP, fluoride varnish, and fluoride gel on the microhardness of permanent teeth enamel.

2- MATERIALS AND METHODS

2-1. Study design and population

This in-vitro study performed on human premolar teeth, which had been extracted for orthodontic purposes. The sample size was chosen according to the Kushki et al.'s studies (33). In the mentioned study, the mean and standard deviation of the desired outcome in the intervention group were 41.07 and 22.21 and in the control group 48.73 and 17.29. By considering these results, confidence interval of 95%, test power of 80%, one-way test, and mean comparison formula, as well as G-power software, it was estimated as 344 totally (86 teeth in each group).

2-2. Methods

After extraction from the oral cavity, the collected teeth were kept inside repositories containing normal saline solution 0.9% (Injection and pharmaceutical products company, Tehran, Iran) (32). Next, the teeth were placed inside formalin 10% solution for two weeks for sterilization. The debris across the occlusal surface of teeth were cleansed through polishing with low-rate handpiece, after washing with normal water, they were placed inside distilled water repositories containers (32, 34). A senior dental student who had received the necessary training performed all stages. A pediatric dentist and a laboratory technician also supervised the project.

2-3. Measuring tools

Next, using a nonstop device Demco (CMP Industries, New York, USA), and two-blade diamond disc, the root of all teeth was cut from 1 mm below as

Cemento Enamel Junction (CEJ), and the crown of the teeth was divided into buccal and lingual parts in the mesiodistal direction (32, 35). The polish of surface of teeth was performed manually and with rotational movements for 1 min using a sandpaper made of silicon carbide (Matador, Aachen, Germany) plus water (32, 36). Also, in the samples in order to increase the assessment accuracy, a 2*2 mm^2 region was determined in the middle one third of the teeth, and covered with a paper pad. The other parts of the crown of teeth were covered with a layer of nail polish (Isadora, Stockholm, Sweden) (32, 37). Then, at the next stage, the samples were evaluated using Vickers microhardness technique (Struers, Willich, Germany) to determine the amount of microhardness. For this purpose, the surface of the samples was subjected to three vertical impacts with a pressure of 300 g for 10 s by a pyramidal diamond. The diameter of each indentation was measured after each impact and the average of these values was reported as the enamel initial microhardness (38).

2-4. Intervention

Next, the samples were placed inside incubator device (Gallenkamp, Munich, Germnay). In order to create demineralization in the teeth enamel, the samples were kept inside a solution with the following formula at 37°C for 48 hours: 2.2 mM calcium chloride, 2.2 mM potassium dihydrogen phosphate, 0.05 mM acetic acid, diluting the volume of the resulting solution to 1 L using distilled water, solution pH adjustment on 4.4 using potassium hydroxide 10 mM solution, and measurement by a pH meter device (32). The samples were investigated by Vickers technique and the resulting number was recorded as the demineralized enamel.

Next, in order to apply the remineralizing materials, the samples were first placed inside artificial saliva in order to simulate conditions of the oral saliva (Kin Laboratory, Madrid, Spain) (39). The remineralizing materials were examined in the following studied groups:

I) Control group: This group received no treatment and was placed inside artificial saliva until the end of the investigation.

II) Fluoride varnish group (Enamelast, Ultradent, USA): A thin layer of varnish (containing sodium fluoride 5%) was smeared on the surface of samples according to the manufacturer's instruction using a micro brush, and then the extra varnish was removed using air syringe.

III) Fluoride gel (Topex 60 second, Sultan healthcare, USA): A thin layer of gel (Acidulated Phosphated Fluoride (APF) containing sodium fluoride 2.59%): It was also smeared on the surface of samples according to the manufacturer's instruction using a micro brush, and then the excess substance was removed using air syringe.

IV) CPP-ACP group (GC Tooth Mousse, GC America, USA): it was smeared on the surface of samples according to the manufacturer's instruction using a cotton applicator of tooth mousse for 3 min, whereby the excess material was removed using clean applicator.

Once smeared with remineralizing materials and after cleaning the excess materials from the surface of sample, groups II-IV were placed inside repositories containing artificial saliva. The mentioned interventions were performed for each group for 14 days twice per day. In addition, the artificial saliva solution was replaced with fresh solution midway half through the test period. After completion of the 14-day period of experiment, the samples very investigated in terms of microhardness of the remineralized enamel (32, 35, 39).

2-5. Inclusion and Exclusion Criteria

The specimens collected in the present study included intact, caries-free permanent premolars that had been extracted for orthodontic reasons. These teeth did not have any caries, cracks, or discoloration.

2-6. Ethical Considerations

Since it was an in vitro study, no special ethical considerations are considered. However, the code of ethics related to the research project has been received from the Ethics Committee of Mazandaran University of Medical Sciences (IR.MAZUMS.REC.1399.8430).

2-7. Data Analyzes

Specimens were assigned to four groups by blocking method. Data analysis was carried out using ANOVA, repeated measures ANOVA, and Friedman tests in SPSS V.25. P-value<0.05 was considered as the significance level.

3- RESULTS

In this study, 344 specimens were studied equally in four groups: I. Control, II. Fluoride varnish, III. Fluoride gel and IV. CPP-ACP group. The mean microhardness values between different groups were evaluated by ANOVA, repeated measures ANOVA, and Friedman tests (**Table.1**, **and Figure.1**). ANOVA test showed a statistically significant difference between different groups in terms of the mean microhardness value in the remineralized enamel section (p<0.001), and no statistically significant difference was observed between the four groups in terms of the initial and demineralized enamel section (p=0.146, and p=0.131, respectively). The mean remineralization values in the studied groups were as follows: Group III> Group II> Group IV> Group I.

The mean values of remineralization, demineralization, and initial enamel in different groups were also analyzed based on Friedman test and results showed statistically significant difference between different groups in this regard (p <0.001), that is, the mean value of Initial enamel > remineralization > demineralization. It should be noted that based on the results of repeated measure ANOVA analysis, the mean values of microhardness at different times between the studied groups are statistically significant (**Figure.1**).

Table-1: Mean comparison of the Vickers microhardness values across the studied groups.

Enamel stage	Group				P-value	
	Group I, n= 86	Group II, n= 86	Group III, n= 86	Group IV, n= 86	ANOVA	Repeated measure ANOVA
Initial	289.47 ± 15.79	287.63 ± 12.16	290.68 ± 15.84	285.91 ± 13.61	0.146	< 0.001
Demin.	170.36 ± 18.64	164.95 ± 14.57	166.49 ± 12.96	168.73 ± 17.97	0.131	
Remin.	178.63 ± 21.09	221.75 ± 12.69	241.21 ± 15.60	211.43 ± 16.04	< 0.001	
P-value (Friedman)	< 0.001	< 0.001	< 0.001	< 0.001	-	

Group I= Control, Group II= fluoride Varnish, Group III= fluoride Gel, Group IV= CPP-ACP.

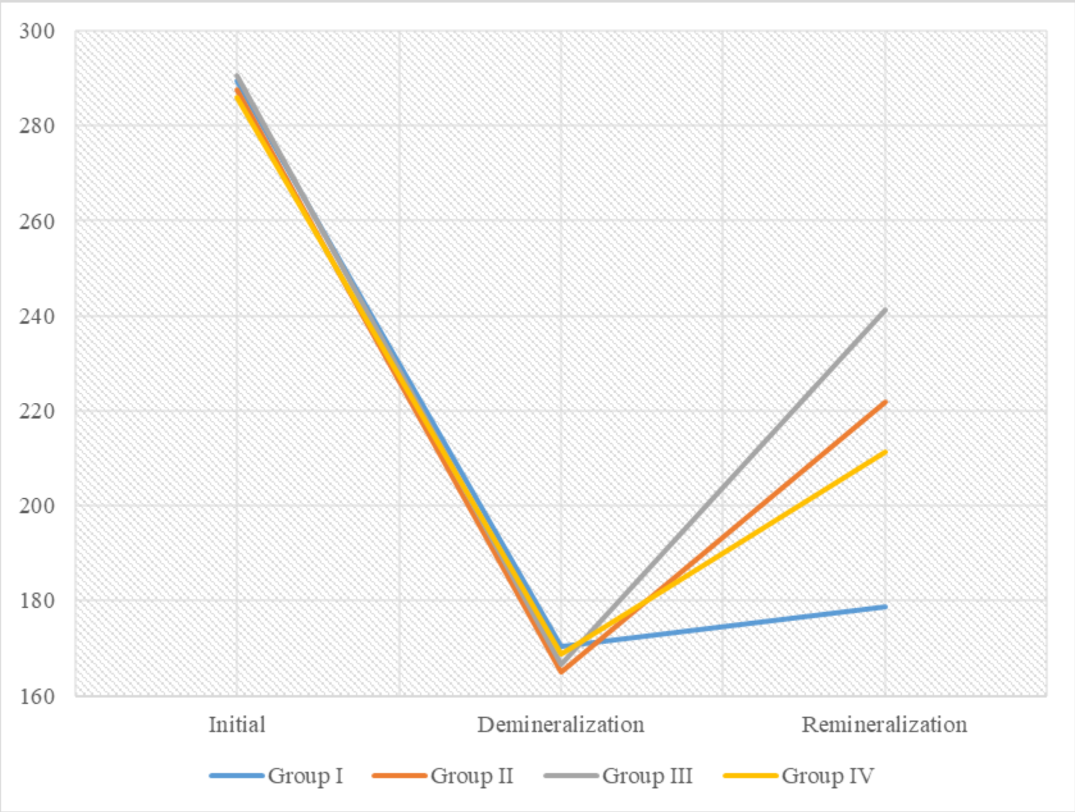

Fig.1: Mean comparison of the Vickers microhardness values at different times across the studied groups.

Group I= Control, Group II= fluoride Varnish, Group III= fluoride Gel, Group IV= CPP-ACP.

4- DISCUSSION

The aim of this study has been to in-vitro investigation the effect of CPP-ACP, fluoride varnish, and fluoride gel on the microhardness of permanent teeth enamel. In the current investigation, dental samples were examined in four groups: 1- control, 2- fluoride varnish, 3- fluoride gel, and CPP-ACP. Among mentioned materials, CPP-ACP showed a weaker performance compared to fluoride-containing groups for enhancing the microhardness of tooth enamel. This is in line with various studies (9, 11, 32). Generally, as with all cases in the healthcare, prevention from dental caries is more important than its treatment. Tackling progressive caries that occur in children and infants is challenging in pediatric dentistry, which sometimes requires treatment under general anesthesia. Accordingly, it is evident that if this disease could be prevented, many of its resulting costs and problems would also be prevented (27). Today, it is believed that aggressive treatment of dental caries alone cannot halt the progression of this disease, and other complementary issues should also be observed for its management (8). Although dental caries seems to be an irreversible disease, there is evidence on its reversibility, though the effective measures should be taken in its primary stages (7, 9-12). Evidently, loss of the teeth mineral tissue known as demineralization followed by diminished enamel hardness play a key role in dental caries, as the dental tissue becomes exposed to environmental factors of the oral cavity with minimum resistance and defense coverage (6). Considering the relationship between mineral content and microhardness of tooth enamel, the latter can function as an index for examining the power of various remineralization materials in halting the progression of demineralization and reversing incipient lesions (27, 40). Sharifi et al. examined and compared CPP-ACP, CPP-AFCP, sodium chloride mouthwash, and sodium fluoride mouthwash plus CPP-ACP. They concluded that CPP-ACP alone had a minor performance in enhancing the microhardness of enamel compared to the other mentioned materials. Also, use of CPP-AFCP known fluoridated CPP-ACP had a relatively greater potential (32).

Byeon et al. also concluded that fluoride-containing supplements significantly enhanced the microhardness properties of CPP-AFCP compared to when the material was used alone (9). Liena et al. also examined the performance of CPP-ACP, CPP-AFCP, and fluoride varnish. However, they found no evidence on the functional superiority of CPP-ACP over varnish. In addition, in the end, the microhardness properties of fluoridated CPP-ACP was greater than those of the two others (11). In their study, Kushki et al. tested CPP-AFCP and varnish fluoride, and concluded that all of the mentioned materials improved the enamel microhardness, though fluoride varnish had a relatively poorer performance (33).

Heravi et al. in their clinical study on examining remineralizing compounds on primary carious lesions observed that treating white lesions using CPP-AFCP and Nano paste caused a significant reduction in the breadth of white lesions, increase in mineral content, and improvement of their appearance. This improvement was better than natural improvement of white stains following daily use of fluoridated toothpaste, though CCP-AFCP was more effective than Nano paste (10). Investigation of the above studies suggests that the synergism of fluoride with CPP-ACP can significantly enhance the enamel microhardness. Presence of fluoride ions in dental cavity results in formation of fluor - hydroxyapatite crystals from the calcium and phosphate ions in saliva. This would cause elevated pH of the oral cavity; this not only provides an acid-resistant environment, but also leads to formation of larger crystals of fluor-hydroxyapatite. Thus, remineralization is accelerated, a resistant surface is also formed, and eventually the resistance of the dental tissue to demineralization increases (41).

Meanwhile, CPP-ACP, containing casein phosphate, leads to stabilization of calcium and phosphate ions, and deploys small molecules of these ions across the tooth surface. Ultimately, these compounds functions as a source for the remineralization (27). Addition of calcium and phosphate ions to the fluoride formulation in CPP-ACP form can enhance fluoride resorption by the tooth enamel (42-44). Examining studies further, the study by Isfahani et al. (27) is one of the few studies mentioning the superiority of CPP-ACP alone compared to fluoride-containing materials. They found that when using the above-mentioned products for a long run, CPP-ACP showed greater potential in enhancing the enamel microhardness properties.

By the long run, they meant four weeks, which has been longer from the two-week period in which the current evaluated samples underwent treatment with remineralizing compounds. Nevertheless, although Llena et al. (11) as well as Huang et al. (30) examined eight weeks, they reported discrepant results compared to Isfahani et al. (27). Generally, the differences in the results reported by various studies can be attributed to the different type of the microhardness measurement devices, investigational conditions including in-vitro or clinical conditions, as well as the type of teeth examined (healthy enamel, lesion with primary caries, etc.). Another notable point is the slight change in the mean remineralized enamel of the control group compared to the demineralized state. This can be due to the artificial saliva content. This material contains substances such as KCl, $MgCl_2$, $CaCl_2$, and $KHSO_4$, which can be effective in enhancing the microhardness and strength of the tooth enamel (32, 45). In the current investigation, the power of fluoride-containing products especially fluoride gel was greater than that of CPP-ACP in enhancing the microhardness properties of the tooth enamel. As mentioned earlier, gel is one of the materials to locally deliver fluoride to the teeth, and since it is used through a tray, all teeth can get into contact simultaneously (23).

The gel used in the current investigation contained sodium fluoride 2.59% of APF type. The point that should be considered here is the pH of the type of gel used. According to evidence, acidic pH significantly increases the extent of accumulation of fluoride-containing products on the tooth enamel (46-48). Hali et al. (49) in their clinical trial study reported that the growth of lactobacillus and Streptococcus mutans microorganisms significantly decreased after consuming APF gel compared to sodium fluoride neutral gel.

On the other hand, Delbem et al. (46) found that APF gel formed higher levels of fluoride on the tooth enamel compared to the sodium fluoride neutral gel. Also, APF was also more effective in reducing the demineralization of the samples. Accordingly, in the current investigation, the acidic nature of the gel used seem to have been effective in enhancing the microhardness compared to the neutral sodium fluoride varnish. Fluoride varnish is another material for which suitable performance has been reported on improving the enamel microhardness properties. Although varnish has disadvantages including temporary discoloration of the teeth or in some cases unpleasant taste and feeling for children (20), since children well tolerate the use of varnish, this material is one of the simplest and most suitable ways of delivering fluoride to the tooth enamel especially in children (19-22). The American Dentistry Association (ADA) has concluded that fluoride varnish with 2.26% concentration is recommended for all children below six years of age who are at high risk of developing dental caries. A meta-analysis in 2016 also showed that local fluoride varnish is effective in preventing the primary lesions of dental caries (50-52).

In the study by Hojjati et al. (53) on comparing the power of APF fluoride gel and varnish on the strengths of tooth enamel against demineralization, it was found that both materials showed good resistance against demineralization, though they were not different with each other. The difference in the type of technique of investigating the level of remineralization in the above study compared to the current investigation can explain the different results. Note that studies evaluating different types of fluoride varnish and gel under in-vitro conditions on the enamel microhardness have been very limited.

Generally, since fluoride in its gel form may be available at different concentrations, it should be applied cautiously. Children undergoing treatment with fluoride gel have complained from nausea, vomiting, headache, and abdominal pain. Accordingly, although the power of this type of fluoride has been evident in the current investigation, its usage by children is not generally recommended (24). Although swallowing CPP-ACP is harmless and it has also a more desired taste compared to other remineralizing materials, and as it well provides the calcium and phosphate reserves of the tooth enamel (27, 31), since according to the evidence obtained in this study, CPP-ACP alone has not shown the sufficient potential of improving the enamel microhardness compared to fluoride containing compounds, accordingly it does not seem to be one of the major remineralizing compounds.

4-1. Study Limitations

The limitations included not investigating the parameters in oral cavity; since the current investigation has been performed under in-vitro conditions, these factors were not recorded or analyzed.

5- CONCLUSION

All of the evaluated remineralizing materials enhanced the tooth enamel microhardness. Nevertheless, fluoride-containing products showed greater potential in improving the microhardness and strength of the tooth enamel compared to the compounds containing CPP-ACP.

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7- CONFLICT OF INTEREST: None.

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