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EFFECT OF CASEIN PHOSPHOPEPTIDE AMORPHOUS CALCIUM FLUORIDE PHOSPHATE VARNISH ON WHITE SPOT LESIONS AND SHEAR BOND STRENGTH OF ORTHODONTIC COMPOSITE: AN IN-VITRO STUDY

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KEYWORDS

CPP-ACP; MI varnish; Orthodontic; Shear bond strength; White spot lesions.

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ABSTRACT

Introduction: Notwithstanding progress in caries prevention methods, averting enamel demineralization during orthodontic treatment remains a significant challenge for clinicians. Orthodontic treatment primarily aims to improve both dental and facial aesthetics, yet protecting the enamel from demineralization continues to be a critical concern. **Aim:** This research aims to assess the enhancements that occurred around orthodontic brackets as a result of the application of Casein Phosphopeptide Amorphous Calcium Fluoride Phosphate (CPP-ACFP) varnish, as well as to investigate the impact that this varnish had on the shear bond strength of orthodontic composite. **Materials and Methods:** For the purpose of this in vitro experiment, enamel specimens were randomly assigned to two primary groups, with each group consisting of twenty specimens. The groups were determined by the surface treatment that was applied to the enamel. In Group I, which served as the control group, there was no surface treatment applied, but in Group II, the surface was treated with CPP-ACFP varnish. After then, each group was divided into two subgroups, each of which contained ten specimens. Subgroup A was subjected to a pH cycling procedure, whilst Subgroup B was immersed in a Streptococcus mutans suspension. **Results**: CPP-ACFP varnish groups showed the greatest resistance to discoloration represented by the least color change without significant effect on shear bond strength. **Conclusions**: Using CPP-ACFP varnish in orthodontic treatment demonstrates potential benefits specifically in reducing white spot lesions and maintaining bond strength.

INTRODUCTION

Clinically, white spot lesions (WSLs) can appear rapidly; they frequently show up as early as the fourth week after orthodontic treatment begins, especially in patients who don't practice good dental hygiene and don't have preventative measures in place [(1)]. The bonding of fixed orthodontic appliances in the oral cavity triggers a significant alteration in the bacterial composition of dental plaque. This shift leads to an increased prevalence of acid-producing bacteria, primarily *Streptococcus mutans* and *Lactobacilli* [(2)]. These bacteria significantly reduced the pH of the plaque in orthodontic patients, causing a more pronounced drop in pH levels compared to individuals not undergoing orthodontic treatment [(3)].

The visual identification of white spot lesions (WSLs) and the assessment of associated risks are crucial steps that must be conducted prior to initiating orthodontic treatment. Detecting WSLs in a patient who has not undergone orthodontic procedures places the individual in a higher risk category for further enamel demineralization. This pre-existing condition suggests a heightened susceptibility to future demineralization during orthodontic therapy, thereby requiring more vigilant preventive measures.

Evaluating for white spot lesions (WSLs) during orthodontic treatment is essential for promptly initiating appropriate management strategies as soon as WSLs are detected. Patients presenting with pre-existing WSLs before the commencement of orthodontic therapy, poor oral hygiene, high intake of dietary sugars, prolonged treatment duration, excessive enamel etching, the use of labial orthodontic appliances, and a high DMFT (Decayed, Missing, and Filled Teeth) or DMFS (Decayed, Missing, and Filled Surfaces) index are classified as high-risk individuals. Additionally, the development of new lesions during treatment further contributes to this elevated risk profile, necessitating increased preventive care and monitoring.

There are a number of therapy protocols that can be utilized for the management of white spot lesions (WSLs). These procedures range from conventional to more intrusive techniques, depending on the degree and breadth of the lesions. Numerous studies have been carried out to develop new strategies for addressing orthodontic-associated WSLs, with a particular focus on early diagnosis and prompt intervention using minimally invasive methods. Early identification and treatment are critical to preventing the progression of these lesions and ensuring better clinical outcomes.

The severity of this widespread problem is highlighted by the fact that the prevalence of white spot lesions (WSLs) varies greatly from study to study, with the range spanning from 33.8% to 97%. It is clear from this variety that there is an immediate and pressing requirement for enhanced knowledge among patients as well as healthcare providers in order to put into practice effective preventive methods. In an ideal scenario, these measures should consist of straightforward, non-invasive, and cost-effective interventions that are simple enough for the patient to carry out without affecting the shear bond strength of the orthodontic brackets. When it comes to decreasing the danger of WSLs during orthodontic treatment and guaranteeing long-term dental health, such preventive treatments are absolutely necessary.

MATERIALS AND METHODS

I. Teeth preparation:

Research was carried out with twenty human premolars that had just been extracted and were in good health. This study was carried out after receiving consent from the Ethical Committee of the Faculty of Dentistry at Suez Canal University (approved number 479/2022). The buccal and lingual surfaces of these premolars were cleaned thoroughly using a rubber cup and polishing paste. The teeth were then inspected under a chairside light and examined with a 3.5x magnification loop to verify the integrity of the enamel. Following confirmation, the teeth were placed in distilled water and the water was refreshed on a weekly basis in order to ensure that they were well hydrated. After that, the teeth were decorated at the cementoenamel junction, and the crowns were sectioned mesiodistally to divide the lingual and buccal halves. This resulted in a total of forty specimens that were subsequently subjected to additional examination.

II. Grouping of the samples:

On the basis of the surface treatment that was given, the enamel specimens were put into two primary groups, with each group consisting of twenty specimens. These groups were randomly assigned to each other. Casein Phosphopeptide Amorphous Calcium Fluoride Phosphate (CPP-ACFP) varnish was applied to individuals in Group II, which was referred to as the varnish group. Group I acted as the control group and did not receive any surface treatment. According to exposing factors each group were further subdivided into two subgroups, each of 10 specimens; subgroup A: were exposed to pH cycling and subgroup B: were immersed in Streptococcus mutans suspension.

Group IA: control group, without surface treatment and exposed to pH cycling.

Group IB: control group, without surface treatment and immersed in Streptococcus mutans suspension.

Group IIA: Treatment group, were treated with varnish and exposed to cycling.

Group IIB: Treatment group, were treated with varnish and immersed in Streptococcus mutans suspension.

III. Steps

1. Pre-bonding intervention:

The enamel specimens from subgroups IIA and IIB were first cleaned and thoroughly dried. A thin, uniform layer of MI varnish was then applied to each specimen, allowing it to set for one minute. Following this, the specimens were incubated in 10 ml of distilled water for a duration of four hours to facilitate and enhance ionic exchange. After the incubation period, the varnish was carefully removed using a periodontal curette to avoid damage to the enamel surface.

2. Bonding:

The bracket placement for both control and treatment groups were done by the same orthodontist following the same bonding protocol.

3. Post-bonding intervention:

A second layer of MI varnish was applied to subgroups IIA and IIB specimens as a 2 mm band all-around the bracket base then enamel specimens were left undisturbed and incubated in distilled water for another 4 hours. Then the varnish was removed gently with periodontal curette.

4. pH cycling:

The enamel specimens from both subgroups I.A and II.A were immersed in a demineralizing solution containing 2.0 mmol/L of calcium, 2.0 mmol/L of phosphate, and 75 mmol/L acetate buffer at a pH of 4.3 for three hours. Following this, they were placed in non-ionized water with a pH of 7.0 for 21 hours. This demineralization cycle was repeated over a period of 30 days. Throughout this process, the specimens were incubated at a constant temperature of 37°C, with both the demineralizing solution and the non-ionized water being refreshed every 24 hours to maintain consistency in the experimental conditions.

5. Assessment:

5.a Change in color:

In order to ensure that an exact numerical value of the tooth shade could be achieved, the VITA

Easyshade® device was set to the bleaching mode. After this, the device was prepared for usage. The first measurement (T0) for both the control and treatment groups was recorded using the VITA Easyshade® device immediately before the bonding of the orthodontic brackets. Subsequent readings were taken at 15 days (T1) and 30 days (T2) and were documented accordingly (Figured 1.A). The shade evaluation was based on a lightness scale ranging from 0 to 100, where a higher L value indicated increased light reflectivity, making the object appear lighter. Conversely, a lower L value signified more light scattering, causing the object to appear darker.

5.b Shear bond strength:

The bond strength was assessed using an Instron™ universal testing equipment equipped with a 1 kN load cell, operating at a crosshead speed of 1 mm/minute. (Figured 1.B)

The SBS values were computed in megapascals by dividing the force by the area of the bracket base (MPa = N/mm^2). An occlusal-gingival force was exerted on the bracket using a blade-end steel rod connected to the crosshead of the universal testing apparatus.

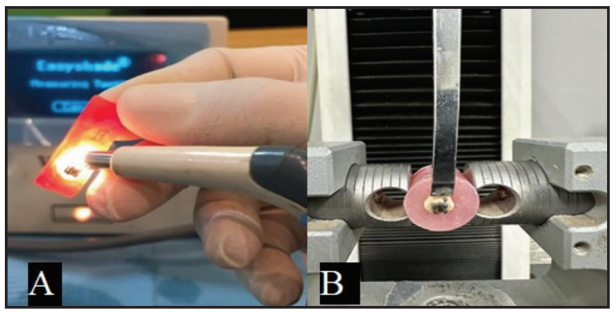

Fig. (1) Assessment of color (A). Measurement of shear bond strength (B)

RESULTS

The data were analyzed using IBM SPSS version 23 (Armonk, NY, USA). Normality was assessed using the Shapiro-Wilk test and Q-Q plots. For the purpose of calculating the percentage change, the following formula was utilized: [(Test Values–Control values Control values) ×100]. The variables, including shear bond strength, changes in enamel shade, and all percent change values, demonstrated a non-normal distribution. As a result, the data were predominantly presented through maximum, minimum, and median values. The Kruskal-Wallis test was utilized for the statistical analysis of non-normally distributed data. Subsequently, Dunn's post hoc test with Bonferroni correction was applied upon the identification of significant differences. A one-way ANOVA, accompanied by Tukey's post hoc test, was utilized to compare calcium levels among the groups. All tests were two-tailed, with statistical significance established at a p-value of less than 0.05.

1. Comparison of enamel shade between the study groups:

Enamel color change descriptive statistics and group comparisons are shown in (Table 1). There was significant difference between groups in both T0-T1 and T0-T2.

Varnish group which exposed to bacteria (Group IIB) showed the greatest resistance to discoloration represented by the least color change either in T0-T1 Or T0-T2 by 1.01 and 2.71 respectively, followed by varnish group which exposed to pH cycling (Group IIA) by 1.36 and 3.05 respectively. In contrast with non-treatment group that exposed to pH cycling (Group IA) which showed greatest color change in T0-T1 2.68, while non-treatment group that exposed to bacteria (Group IB) showed greatest color change in T0-T2 11.84.

2. Shear bond strength of study groups:

The descriptive statistics for the shear bond strength (SBS) of orthodontic brackets, along with the group comparisons, are presented in **Table 2**. Varnish group which exposed to bacteria (Group IIB) resulted in highest mean SBS value 16.42 Mpa followed by varnish group which exposed to pH cycling (Group IIA) 14.66 Mpa, then nontreatment group that exposed to bacteria (Group IB)12.88 Mpa and finally non-treatment group that exposed to pH cycling (Group IA) showing the lowest mean SBS value 10.35 Mpa. There was no significant difference in SBS between test groups (P = 0.164).

Table (1) *Comparison of the change in enamel shade between the study groups*

		Group IA (n=10)	Group IB (n=10)	Group IIA (n=10)	Group IIB (n=10)	(*P* value)
ΔT0–T1	**Mean ± SD**	2.68 ± 1.77	1.15 ± 0.84	1.36 ± 2.18	1.01 ± 0.87	(0.039*)
ΔT0–T2	**Mean ± SD**	10.48 ± 3.83	11.84 ± 3.79	3.05 ± 1.38	2.71 ± 1.85	(<0.0001*)

**Statistically significant difference at p value≤0.05*

Table 2: Comparison of shear bond strength among study groups

	Group IA (n=10)	Group IB (n=10)	Group IIA (n=10)	Group IIB (n=10)	(*P* value)
Mean ± SD	10.35 ± 2.61 Mpa	12.88 ± 5.84 Mpa	14.66 ± 6.79 Mpa	16.42 ± 6.23 Mpa	(0.164)

*Mpa: Mega Pascal *Statistically significant difference at p value≤0.05*

DISCUSSION

Traditional methods of biofilm removal, such as tooth brushing, dental flossing, and antiseptics, rely on patient compliance, which can be challenging to achieve. Moreover, the use of antiseptics can contribute to the development of drug-resistant microbes and may result in various other side effects [4].

As the adage goes, "prevention is better than cure," the importance of a preventive approach during orthodontic treatment has become evident. Different methods of fluoride application have proven effective in preventing the formation of white spot lesions (WSLs) [5,6].

Casein Phosphopeptide-Amorphous Calcium Fluoride Phosphate (CPP-ACFP), marketed as MI Varnish™ , was selected as the treatment material for this study. This varnish is translucent, stable, commercially available, and easy for patients to self-apply. The maker claims that it is formulated with 5% sodium fluoride (NaF) and augmented with 2% RecaldentTM (CPP-ACP), which is generated from casein phosphopeptides (CPP) that are naturally present in milk. The component known

as Amorphous Calcium Phosphate (ACP) functions as a source of phosphate and calcium, releasing fluoride, phosphate and calcium ions that are highly bioavailable. Fluoride ions preferentially interact with the peptide that is produced when fluoride ions come into contact with CPP-ACP. This results in the production of an optimal source of ions that are necessary for the synthesis of fluorapatite, which is a more durable form of enamel.

The CPP-ACFP particles have a size of less than 2 nanometers, allowing them to penetrate biofilms, enamel, and dentin effectively. Due to its neutral charge, its diffusion properties are not impeded. The varnish is formulated in a hydrogenated resin matrix combined with an ethanol solution, enabling it to adhere securely to enamel, dentin, and oral mucosa. The fluoride and CPP-ACP are released in a regulated manner when the varnish is exposed to saliva, which causes it to harden and initiate a slow disintegration process.

The formation of calcium fluoride globules allows the fluoride ions released from the varnish to effectively bind with the calcium ions found in the pellicle and dental plaque. When these globules eventually settle on the surface of the tooth, they contribute to the occlusion of the micropores in the enamel as well as the tubules in the dentin. The remineralization of enamel is facilitated by this process, which not only increases the tooth's resistance to acid but also fills saliva with calcium and phosphate at the same time.

MI varnish has been previously assessed in several studies comparing its efficacy with other fluoride-releasing agents. For instance, **Rechmann *et al.*** [7] evaluated the effects of daily application of MI Paste Plus alongside quarterly MI varnish treatments on the incidence of white spot lesions (WSLs) during fixed orthodontic treatment. Similarly, Shen et al. [8] and Mishra et al. [9] conducted studies that explored the effectiveness of MI varnish in preventing the development of WSLs, comparing it with alternative fluoride delivery systems.

The subsurface demineralization process, which begins with the formation of pores between the enamel rods, is the first step in the disintegration of enamel crystals. As a result of the disparity in refractive indices (RIs) that exists between demineralized and undamaged enamel, the demineralized regions have a whitish appearance [10]. The existence of microporosities is the reason of this shift in color that occurs in the enamel that is affected. Because these pores are filled with either water (RI = 1.33) or air (RI = 1.0), the enamel appears opaque when compared to healthy enamel, which has a refractive index of 1.62. This is because the pores are filled with either water or air. As a result of the pores becoming filled with air, the lesions become more noticeable as a result of the increased light scattering that occurs when the enamel is dried out. The difference in refractive indices between the enamel crystals and the media that is contained within the pores is directly responsible for the whitish opacity that is exhibited by these diseases [11].

Hence, the change in color can serve as a reliable indicator for evaluating the efficacy of white spot lesion prevention or treatment [12]. spectrophotometer was used in this study. Research have demonstrated that using a spectrophotometer for color change evaluation is more reliable than the subjective assessment of shades using various shade guide systems [13].

In this study, the mean color change observed in the control subgroups, which did not undergo any treatment, was (2.68 ± 1.77, 1.15 ± 0.84) after 15 days and (10.48 ± 3.83, 11.84 ± 3.79) after 30 days. In comparison, the treatment group that received MI Varnish™ showed a mean color change of

(1.36 ± 2.18, 1.01 ± 0.87) after 15 days and (3.05 ± 1.38, 2.71 ± 1.85) after 30 days. These results demonstrate that the degree of demineralization in the control subgroups over the 30-day period was significantly greater than in the subgroups treated with MI Varnish™ .

In a similar vein, there was no statistically significant difference between the subgroups of treatment in terms of the color change results after 15 and 30 days. However, a statistically significant contrast was observed within the control subgroups between color change results at 15 days and 30 days. These findings suggest that the degree of demineralization significantly increased from the 15-days follow-up interval to the 30 days follow-up period within the control subgroups, in contrast to the treatment subgroups, which means that MI varnish has significant resistance to enamel discoloration in both the presence of acidogenic bacteria or acidic change.

The findings from our study align with prior research that investigated the preventative capabilities of MI varnish™ [14,15]. CPP-ACFP was evaluated by **Abufarwa *et al.*** [16]. Mishra et al. [17] found that the CPP-ACFP value was superior to all other groups. They compared the efficacy of CPP-ACP, CPP-ACFP, fluoride mouthrinse, and fluoride-containing toothpaste against a control group for the remineralization of white spot lesions (WSLs) in orthodontic patients with fixed appliances.

To clear the conflicting findings that emerged regarding the impact of applying fluoride topically on the strength of bracket bonds, one of this study objectives was to assess the impacts of applying CPP-ACFP topically on the shear bond strength.

Treatment subgroup which exposed to bacteria (subgroup IIB) resulted in highest mean SBS value 16.42 MPa. While non-treatment nongroup that exposed to bacteria (subgroup IB) showed SBS of 12.88 MPa.

In addition, treatment subgroup which exposed to pH cycling (subgroup IIA) showed 14.66 MPa, whereas non-treatment subgroup that exposed to pH cycling (subgroup IA) showing the lowest mean SBS value 10.35 MPa. Despite there was no significant difference in SBS between study groups, the findings revealed that the application of MI varnish either before enamel etching or after bracket bonding did not hinder the bracket SBS. This was in agreement with **Tabrizi *et al.*-**[18] and **Baysal *et al.*** [19]

Limitations

While our study demonstrated promising results regarding the effects of CPP-ACFP varnish, it is important to acknowledge some limitations. Firstly, the in-vitro nature of the study may not fully capture the complex oral environment and the dynamic interactions between the treatment agents and oral tissues. Therefore, the findings should be interpreted with caution and validated through clinical trials involving human subjects.

Additionally, the concentration and application protocols of CPP-ACFP varnish used in this study were based on established guidelines and previous research.

Moreover, the study focused on the antimicrobial effect on Streptococcus mutans only while neglecting other cariogenic bacteria in the oral cavity.

The results may be affected by differences between the buccal and lingual enamel surfaces of the premolars such as enamel thickness and roughness. Furthermore, the orthodontic brackets used in the study were designed to fit the buccal contour of premolars and not the lingual ones. Considering that in the oral cavity the self-cleansing capabilities of the lingual surface are higher than the buccal surfaces.

CONCLUSION

In conclusion, our study demonstrates the potential benefits of CPP-ACFP varnish in orthodontic treatment, specifically in reducing white spot lesions and maintaining bond strength. However, further research is warranted to confirm and expand upon these findings, considering the limitations of this study. By continually exploring and refining preventive strategies, orthodontic treatment can be optimized to promote oral health and enhance treatment outcomes for patients.

Suggestions

1. Using CPP-ACFP varnish in during orthodontic treatment is beneficial in controlling white spot lesions.
2. It is worth considering different concentrations or application techniques in future studies.
3. For further studies, other factors such as esthetics, patient comfort, and long-term stability of the treatment outcomes should also be considered and could incorporate comprehensive assessments that encompass a broader range of clinical parameters.

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