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Color alterations in teeth with orthodontic treatment exposed to pigmenting substances, with and without fluoride varnish protection with tricalcium phosphate: An *in vitro* study

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Abstract
Introduction: At the end of orthodontic treatment, it is common to observe dental color alterations, making the teeth appear more yellowish or stained. This often leads to tooth whitening procedures, which are invasive treatments unsuitable for patients with dental sensitivity. This study proposes a preventive protocol using Clinpro™ White Varnish with tricalcium phosphate fluoride varnish after bracket cementation, which is easy to apply and manage, to reduce demineralization and dental pigmentation.
Materials and Methods: This was an *in vitro*, experimental, and longitudinal study. Eighty healthy premolars extracted for orthodontic reasons were bracketed and divided into two groups of 40: one without enamel protection and another with protective varnish. Each group was subdivided into four subgroups (10 premolars each), which were submerged in Coca-Cola®, coffee, dark beer, or a control group for 10 days. The color was evaluated before cementation and after bracket debonding through digital photographs using Adobe Photoshop 2022.
Results: All teeth exposed to pigment substances showed significant color changes, most notably in the unprotected group. The protected group presented higher L values, indicating whiter teeth. Kruskal-Wallis tests revealed significant differences in Coca-Cola® (P=0.0007), coffee (P=0.0007), and beer (P 0.0043).
Conclusion: Clinpro™ White Varnish fluoride varnish significantly reduces postorthodontic dental pigmentation, promotes remineralization, and improves dental color naturally, without invasive procedures.

Keywords: Dental pigmentation, fluoride varnish, orthodontics

Introduction
A large percentage of orthodontic treatments are initiated by patients for aesthetic reasons, as they are unaware of the presence of malocclusions and the functional consequences these may cause. However, a frequent issue upon completion of treatment is the change in the appearance of the dental color, which may turn darker, yellower, or lead to the development of visible stains. This situation leads to dissatisfaction among patients, who then opt for complementary treatments such as teeth whitening, which, while improving appearance, may expose the dental structure to potentially harmful chemicals.
Importantly, not all patients are candidates for tooth whitening, which limits the alternatives available to improve dental color perception.
One critical moment when teeth are exposed to chemical substances is during the placement of fixed orthodontic appliances. This process includes acid etching and adhesive application, which can demineralize the enamel surface, generating porosities and increasing susceptibility to the absorption of pigmenting substances.
Additionally, common dietary items, such as coffee, cola drinks, and dark beer, not only favor dental pigmentation but also alter the salivary pH, potentiating negative effects on the enamel.

Moreover, the interaction between the oral environment and the metals and materials used in brackets may contribute to corrosion and, consequently, influence dental color change. Given the multiple factors that promote enamel demineralization and pigmentation, it is crucial to develop less invasive alternatives that mitigate these adverse effects and restore the oral environment balance, avoiding more aggressive treatments such as teeth whitening.

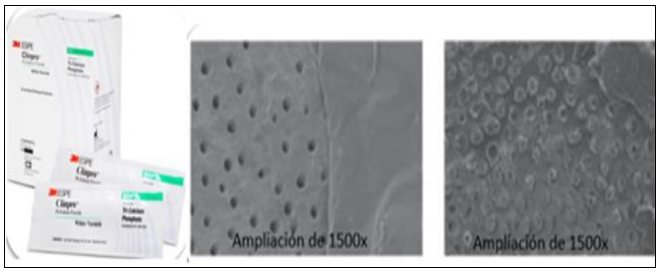
This study proposes the implementation of a new preventive protocol, applying fluoride varnish with tricalcium phosphate (Clinpro™ White Varnish) after bracket cementation, which is easy to apply and handle for both the dentist and the patient, thus reducing demineralization and dental pigmentation (Figure 1).

The objective of this study was to evaluate the efficacy of fluoride varnish with tricalcium phosphate in reducing the color alterations that enamel undergoes during orthodontic treatment from commonly consumed pigmenting substances. Additionally, color alterations were assessed in teeth immersed in coffee, cola, and dark beer for 10 days.

Materials and Methods

In vitro, experimental, and longitudinal studies. Eighty healthy premolars extracted for orthodontic reasons were bracketed. The extracted teeth were placed in saline solution to maintain an environment as close as possible to the oral cavity (Figure 2). When cleaning them, periodontal curettes were used to remove remnants of the periodontal ligament, and the apices were sealed with flow able resin to prevent the solution from infiltrating through this canal. The teeth were then placed back into saline solution for preservation (Figure 3). A bracket was cemented at the center of the crown of each tooth via Ultra Etch acid and Trans bond resin and adhesive from 3M Unitek (Figure 4). Each subgroup was subsequently immersed in one of the three pigment substances and a control group for 10 days (Figure 5). Two color evaluations were conducted: one before bracket cementation and one after debonding. Color evaluation was performed via digital photography taken with a professional camera system (Nikon 7200), an R1C1 flash, and a Nikon 105 mm macro lens. No reflectors were used to avoid altering the color; direct flashes to the object were employed. Images were captured in RAW format obtained from a DSLR camera with automatic white balance calibration. Adobe Photoshop 2022 was used for image processing, ensuring compatibility with the color space for standardized image acquisition for subsequent analysis. All the photographs were taken by the same operator, with standardized settings on the same camera, light conditions, and flash at the same distance to minimize bias.

For color evaluation, an average area of the tooth was chosen, specifically the center of the vestibular face of each tooth, as it would not be directly exposed to the pigmenting substances due to the bracket coverage. This area was selected to ensure that any color change would be due to absorbed pigmentation and not simply staining of the enamel surface. When the image in the software, was opened the white balance was calibrated automatically and accepted. The area to be studied was cropped, and the image was zoomed to 100% for better visualization. The area was marked with a grid tool for more precise measurement, and the color values were extracted via the eyedropper tool to obtain the average L value of the selected area (Figure 6).



Source: Internal data from 3M ESPE

Fig 1: Dentin tubules before and after using Clinpro™ White Varnish.

Fig 2: Teeth in saline solution.

Fig 3: The teeth were cleaned with curettes, and the apex was sealed with resin

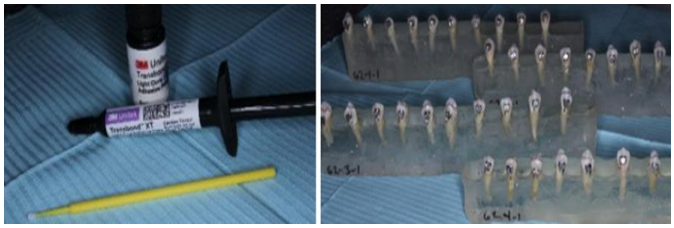


Fig 4: Etching, bonding, and bracket cementation

Fig 5: Groups submerged in pigment substances and the control

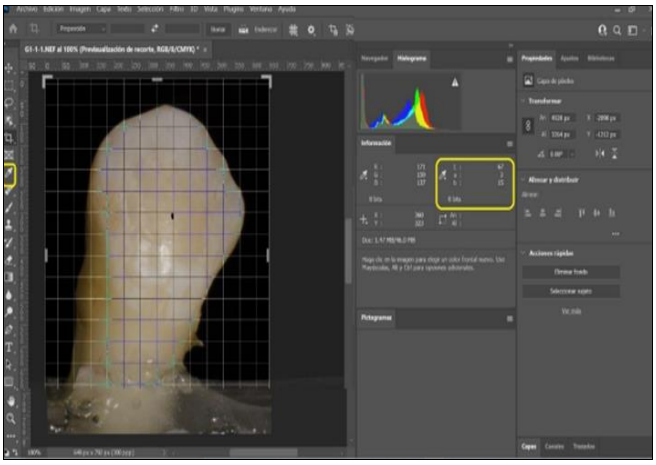


Fig 6: L-Value measurement.

The selected substances are the most commonly consumed substances on the market and are used at room temperature, as controlling other temperatures for 24 hours is not feasible.

- **Coffee:** Nescafé® Classic. Five cups of coffee were prepared to fill the container in which the teeth would be submerged. For each cup, one tablespoon (15 g) of instant coffee was used.
- **Cola drink:** Coca-Cola®.
- **Dark beer:** Victoria® Beer.
- **Control group:** Saline solution with 0.9% (w/v) sodium chloride in water.

Each substance was replaced every 24 hours to prevent bacterial colonization and maintain their properties.
The sample was divided as follows:

Group 1 (G1): Without protective varnish (40 premolars).

- **Group 1-1 (G1-1):** Regular cola drink: Coca-Cola®.
- **Group 1-2 (G1-2):** Coffee: Nescafé® Classic. Five cups of coffee were prepared to fill the container in which the teeth would be submerged. For each cup, one tablespoon (15 g) of instant coffee was used.
- **Group 1-3 (G1-3):** Dark beer: Victoria® Beer.
- **Group 1-4 (G1-4):** Control group: Saline solution.

Group 2 (G2): With Clinpro™ White Varnish protective varnish (40 premolars).

- **Group 2-1 (G2-1):** Cola drink: Coca-Cola®.
- **Group 2-2 (G2-2):** Coffee: Nescafé®.
- **Group 2-3 (G2-3):** Dark beer: Victoria® Beer.
- **Group 2-4 (G2-4):** Control group: Saline solution.

The detailed protocol for each group is described below. (Tables 1-4).

Table 1: Protocol after cementation

Protocol after cementation, First Group	
	Immerse each group in artificial saliva for 24 hours
	Immerse each subgroup in the corresponding substance:
G1-1	10 premolars submerged in Cola soda
G1-2	10 premolars submerged in Coffee
G1-3	10 premolars submerged in Dark Beer
G1-4	Control Group:10 premolars submerged in saline solution

The second group was applied Clinpro™ White Varnish as a protective coating on the enamel after bracket cementation to avoid altering the adhesion process.

Table 2: Protocol to follow during liquid change first group

Protocol to follow during liquid change	Substance change every 24 hours for 10 days
	Rinse each group with saline solution
	Immerse each group in artificial saliva for 25 minutes
	Reintroduce each group into the corresponding substance

Table 3: Protocol after cementation

Protocol after cementation Second Group Application of Clinpro™ White Varnish	
Immerse each group in artificial saliva for 24 hours to allow fluoride release	
After 24 hours, start immersing each subgroup in the corresponding substance:	
G2-1	10 premolars submerged in Cola soda
G2-2	10 premolars submerged in Coffee
G2-3	10 premolars submerged in Dark Beer
G2-4	Control Group:10 premolars submerged in saline solution

Table 4: Protocol to follow during liquid change in the second group

Protocol to follow during liquid change	Change the substance every 24 hours for 10 days
	Rinse each group with saline solution.
	Dry each dental organ and apply Clinpro on the buccal surfaces, surrounding the bracket.
	Let the protective agent act for 5 minutes.
	Submerge each group in artificial saliva for 20 minutes.
	Place each group back into the corresponding substance.

Results

Upon exposure to the cola drink, a difference was observed in the samples after being subjected to the pigmented substances. Group 1 had a median value of 2.00, whereas group 2 had a median value of 7.00. This finding indicated that the group without any enamel protection experienced noticeable darkening of the teeth, whereas in group 2, where Clinpro™ White Varnish was applied, the teeth not only did not darken but whitened. Furthermore, when comparing the groups in the second color measurement, we observed a maximum value of 69 in group 1, whereas the group with Clinpro™ White Varnish had a value of 68. With respect to the averages, Group 1 had a value of 63.7, whereas Group 2 had a value of 61.6 (Table 5).

Table 5: Comparison by groups after being exposed to the cola drink as a pigment.

	Mean ± SD	Minimum	Maximum
Second color take group 1	63.7 ± 3.06	58	69
Second color take group 2	61.6 ± 3.53	55	58

The data are represented in values of L

In the case of coffee, a discrepancy was observed in group 1 after being exposed to the pigmenting substance, with a median of -3.00. On the other hand, the median observed in group 2 was 8.50.

This finding revealed that the teeth submerged in coffee without a protective base darkened noticeably, whereas the group submerged with the application of Clinpro™ White Varnish not only did not darken but actually whitened. In terms of group comparison, similar results were observed in

the second measurement, with group 1 showing an average of 63.7, whereas group 2, which was treated with Clinpro™ White Varnish, had an average of 63.7. In the case of Group 1, the maximum value was 67, whereas the maximum value for Group 2 was 66 (Table 6).

Table 6: Comparison by groups after exposure to coffee as a pigment

	Mean ± SD	Minimum	Maximum
Second color take group 1	63.7 ± 2.31	59	60
Second color take group 2	63.7 ± 2.06	67	66

The data are represented in values of L.

Discussion

The results of this study are similar to those published by Tuncer et al. in 2018, as they mention that the chemical process performed before bracket cementation influences dental pigmentation. They studied the effects of rebonding brackets on dental color, taking into account the possible times brackets may fall off during orthodontic treatment. In their protocol, brackets were cemented, and the teeth were then submerged in pigmented substances and subjected to 24 hours of artificial photo aging. This process was repeated three times, resulting in the conclusion that orthodontic treatment significantly influences tooth color changes. The group that underwent the third phase of the process presented more pigmentation than the initial groups did, as the group without brackets did not significantly differ from the other groups [9].

In the present study, statistically significant differences were detected via the Kruskal-Wallis test in all the groups exposed to pigmented substances compared with the control group; in the cola drink, the p value was 0.007; in the coffee group, the p value was 0.0023; and in the dark beer group, the p value was 0.0043. The first group, which did not have a protective base, presented more pigmentation, whereas the second group, which was treated with fluoride varnish, presented higher L values, resulting in whiter teeth than at the beginning of the study. These results are in line with those obtained in the research project by Mestanza et al. in 2020. In that study, 40 premolars were submerged in different pigmented substances to assess their color change, and they reported that all the substances caused changes to the dental surfaces [2]. Likewise, in 2014, Acosta-Valderrama and colleagues reported that color changes in teeth are inevitable because of the structure of the tooth tissues and the components of various substances [10].

Conclusion

In all the study groups that did not receive any fluoride phosphate tricalcium protective base, significant changes were observed in the reduction of the L value, making the teeth darker or more pigmented. In all study groups where Clinpro™ White Varnish was applied, significant changes were observed in the increase in the L value, resulting in the teeth being whiter.

The study concluded that the use of fluoride varnish with tri-calcium phosphate (Clinpro™ White Varnish) provides significant benefits in reducing pigmentation throughout orthodontic treatment, helping to remineralize enamel and naturally make teeth whiter without invasive procedures. Additionally, the many benefits reported by 3M make it an excellent option. A good practice would be to implement this product (Clinpro™ White Varnish) as a routine step in the adhesion process after bracket placement, with several applications during the treatment. One suggestion would be to perform a control cleaning every 4 months and apply the varnish after the procedure.

Conflict of Interest
Not available

Financial Support
Not available

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