Research Article

An In Vitro Comparison of Clinpro™ XT and Duraphat Varnish for Protecting Teeth from Discoloration during Orthodontic Treatment

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Received 24 September 2022; Revised 6 January 2023; Accepted 9 January 2023; Published 8 February 2023

Objective. During orthodontic treatment, a higher caries risk has been reported, so fluorides and other remineralizing compounds have been proposed. Currently, fluoride varnish is commonly available in conventional and light-curable forms. This in vitro study was performed to evaluate the effectiveness of two different materials, Clinpro™ XT (light-curable forms) and Duraphat (conventional forms) varnish, whose main active principle according to their manufacturers is fluoride in preventing tooth discoloration during orthodontic treatment with appliances attached with metal brackets or resin.

Materials and Methods. This study included 120 premolars free of white spot lesions and caries that were randomly divided into the following six groups (n = 20): resin control (RCTR), resin Clinpro (RC), resin Duraphat (RD), bracket control (BCTR), bracket Clinpro (BC), and bracket Duraphat (BD). All the samples were exposed to 180 ml of coffee (4 times × 5 min/day) for 7 or 28 days. When not immersed in coffee, the teeth were stored in artificial saliva, which was replaced every day. The lightness (L*), red/green axis (a*), and yellow/blue axis (b*) values were recorded with a dental spectrophotometer (Easy shade Advance 4.0) on days 0 (baseline), 7, 14, and 28. The color difference (ΔE*) on days 7 and 28 was also calculated. Results. The data were statistically analyzed using one-way ANOVA and Bonferroni’s test (α = 0.05). Significant differences were observed between the ΔE*, L*, a*, and b* values of the Clinpro™ XT and Duraphat groups and those of the control groups after 7 and 28 days regardless of the attachment type (P < 0.001). On day 7, no significant differences were observed between the Clinpro™ XT and Duraphat groups, whereas on day 28, the Duraphat groups with metal and resin attachments exhibited significant differences from the Clinpro™ XT groups (P < 0.001). No significant differences correlated to the attachment type were observed throughout the discoloration procedure. Conclusions. The application of fluoride varnish during orthodontic treatment can significantly reduce tooth discoloration caused by the staining solution. Clinpro™ XT varnish showed a longer period of efficacy in protecting teeth from discoloration than Duraphat. No differences in tooth discoloration were observed between the groups with fixed and clear appliances.

1. Introduction

Aesthetics is one of the cornerstone objectives of orthodontic treatment with both fixed and clear appliances. Although orthodontic outcomes can be acceptable, several adverse effects of orthodontic treatments have been reported, such as gingivitis, loss of periodontal attachment, white spot lesions (WSLs), and tooth discoloration [1]. During orthodontic treatment, a higher caries risk has been reported, so fluoride [2] and other remineralizing compounds [3] have been proposed. Many patients have complained of tooth discoloration during orthodontic treatment, which can significantly affect the attractiveness of a smile [4].
The color of teeth can be influenced by a combination of intrinsic and extrinsic staining [5]. Direct extrinsic staining, such as that caused by coffee, tea, and red wine, which are commonly consumed, occurs via incorporation of the staining solution into the pellicle; the color imparted is determined by the basic color of the staining solution. Furthermore, some acidic beverages might influence the surface microhardness [6], which increases enamel porosity and consequently increases susceptibility to further staining via adsorption [7]. The accumulation of plaque around complex appliances has been shown to increase due to the difficulty in maintaining oral hygiene throughout orthodontic treatment, which leads to a potential risk of extrinsic staining. Indirect extrinsic staining has been shown to be related to cationic antiseptics and metal salts, which can cause chemical reactions on the tooth surface [8]. Orthodontic metal brackets comprise various metals, and their corrosion might release large amounts of nickel and chromium ions, resulting in enamel discoloration [9]. The resin used to attach clear appliances for orthodontic treatment can preclude the problems caused by the release of metal ions. Currently, no studies have been conducted on the effects of using resin for appliance attachment on tooth discoloration compared to those of using metal brackets.

The best strategies for the management of tooth discoloration seem to be those that prevent its occurrence. Currently, fluoride plays an important role in mitigating acid attacks [10]. The layer formed by fluoride varnish on the tooth surface can even act as a shield against tooth discoloration caused by food and drink. However, the different forms of fluoride agents, such as fluoride toothpaste, fluoride gel, and tooth protectors, usually are ineffective. This inefficiency is often associated with the low fluoride concentrations in the products as well as low patient cooperation [11, 12]. Professional fluoride varnish is the preferred method of topical application because of its simplicity and use of fluoride and lack of patient dependency. Currently, fluoride varnish is commonly available in conventional and light-cureable forms. Conventional fluoride varnish is widely used; however, it must be reapplied every three months to maintain its effectiveness [13]. Recently, a light-cureable resin-modified glass ionomer that can continuously release fluoride and calcium phosphate has attracted widespread clinical attention [11]. Moreover, the manufacturer claims that this light-cureable varnish can provide a protective coating for up to six months, with the potential for more controlled and sustained fluoride release [14].

According to the manufacturers, the chemical composition of the two materials was different, which are listed in Supplemental Table 1. To provide clinicians with better medication guidelines, it is necessary to study the effects of these two forms of fluoride varnish on protecting teeth from discoloration. Moreover, a comparison of these two forms when orthodontic appliances are attached with resin and brackets has not been performed. Therefore, the following null hypotheses were tested: (1) these two forms of fluoride varnish are not effective in preventing tooth discoloration during orthodontic treatment; (2) these two different fluoride varnishes do not show any significant differences in protecting teeth from discoloration during orthodontic treatment; and (3) there are no significant differences in tooth discoloration during orthodontic treatment between appliances attached with metal brackets and those attached with resin.

2. Materials and Methods (Supplemental Figure 1)

2.1. Sample Preparation. A total of 140 human premolars were obtained from orthodontic patients aged 12–15 years old (Supplemental Figure 2A). The inclusion criteria for the teeth were as follows: (a) no dental caries, dysplasia, cracks, erosion, or discoloration; (b) no history of dental treatment; and (c) a storage time less than 30 days. The following exclusion criteria were applied: (a) active caries, enamel demineralization, fluorosis staining, or heavy restorations; (b) previous orthopedic/orthodontic treatment; (c) extraction from a smoker; and (d) a storage time longer than 30 days. According to the inclusion criteria and sample size calculation (power, 0.9; α, 0.05; and effect size, 0.40), 120 teeth were included in the experiment. After extraction, the enamel of the premolars was cleaned with a rubber cup (TPC, Advanced Technology, California, USA) at slow speed for 5 seconds [1]. Half of the roots of the premolars were sectioned and discarded, and after the pulp was extracted with a pulp needle, the remaining crowns were soaked in artificial saliva in a water container at 37°C during the entire study so that any effects of temperature and lighting were eliminated [15]. Fresh artificial saliva was prepared every day.

2.2. Sample Size Calculation. G-power software (University of Düsseldorf, Düsseldorf, Germany) was used to estimate an appropriate sample size. The power of the primary outcome (ΔE∗) was calculated based on our previous pre-experiment with a power of 0.9, an effect size of 0.40, and an α of 0.05. The results indicated that a minimum sample size of 19 teeth was required per group. The final sample size was determined to be 20 teeth per group, which meant that 120 teeth were required for our study.

2.3. Experimental Design. The teeth were randomly assigned a number from 1 to 120 and randomly divided into six groups with 20 teeth each (Figure 1). The six groups were established as follows: resin control (RCTR): bonded with resin, no fluoride applied; resin Clinpro (RC): bonded with resin, Clinpro™ XT varnish applied (Clinpro™ XT; 3M ESPE, Pymble, New South Wales, Australia); resin Duraphat (RD): bonded with resin, Duraphat varnish applied (Duraphat™, Colgate-Palmolive, New York, USA); bracket control (BCTR): bonded with brackets, no fluoride applied; bracket Clinpro (BC): bonded with brackets, Clinpro™ XT varnish applied; and bracket Duraphat (BD): bonded with brackets, Duraphat varnish applied.

The root of each tooth was embedded in silicone rubber impression material (Supplemental Figure 2B).
Two pairs of customized plastic trays with windows 6 mm (Supplemental Figure 2c2) or 3.5 mm (Supplemental Figure 2c3) in diameter and located in the middle of the buccal crown surface of each tooth were fabricated with a 1.0 mm-thick soft-tray sheet according to the sample tooth crowns. The 3.5 mm-diameter windows were used to limit the range of acid etching. The 6 mm-diameter windows were used to ensure that spectrophotometric readings were obtained with a spectrophotometric probe (VITA Classical shade guide, VITA-Zahnfabrik, Bad Säckingen, Germany) at the same sites [16]. Groups of resin attachments (Supplemental Figure 3A) were used to make alginate impressions (Supplemental Figure 3B) and filled with white plaster (Supplemental Figure 3C). The resin attachment on the buccal side of the plaster model was formed with light-cured composite resin (Z350, 3M, São Paulo, USA), with a thickness of approximately 1.5 mm. Then, the attachment templates (Supplemental Figure 3F) of these groups were fabricated with a 1.0 mm-thick hard-tray sheet.

The Ethics Committee of the School of Stomatology at Fujian Medical University reviewed and approved the methodological protocol for this research and ensured that all the procedures met the guidelines of the Medical Ethics Committee (2019-IRB-35).

2.4. Adhesion Procedure. Before adhesive application, all teeth were cleaned with a rubber cup at slow speed for 5 seconds, rinsed, and dried [1]. The following adhesion procedures were then performed:

RCTR group: first, the teeth were placed in a transparent retainer with 3.5 mm-diameter windows. The exposed enamel was etched with 37% orthophosphoric acid for 15 seconds, rinsed with air-water spray for 30 seconds, and dried for 10 seconds. Then, the resin attachment template filled with nanohybrid composite resin (Z350, 3M, São Paulo, USA) was placed on the teeth with adhesive resin (Transbond XT, 3M unit, São Paulo, USA). The resin attachment was cured vertically for 20 seconds, with the light distance not exceeding 2 mm. Then, the crowns were immediately soaked in artificial saliva.

RC group: Clinpro™ XT varnish was added to the appliance
RD group: the appliance was supplemented with Duraphat varnish
BCTR group: MBT metal premolar brackets were bonded with the same bonding system as in the RCTR group.
2.5. Coffee Cycling. The teeth were soaked in 180 ml of Nestle instant black coffee (Nestle Switzerland, Vevey, USA) for 5 min (Supplemental Figure 2D) 4 times per day between 8 a.m. and 5 p.m. The interval between each coffee soaking was 2 hours (Supplemental Figure 4). When not soaked in coffee, these teeth were immersed in artificial saliva, which was replaced every day.

2.6. Debonding and Resin Removal. On days 7 and 28 of the study, 10 teeth in each group were removed from artificial saliva separately. The metal brackets were removed gently with a debonding plier. The resin attachments and residual resin were removed by cleaning with a tungsten carbide bur under loupe magnification (3.3x) until no residual resin was observed with the naked eye. Then, a rubber cup was applied at slow speed for 5 seconds [1]. Afterward, color measurements were performed in the same environment.

2.7. Color Assessment. Prior to the color measurement, the enamel was cleaned with a rubber cup at a slow speed and then thoroughly rinsed with running water. All teeth were inserted into the corresponding silicone rubber base and placed in the transparent retainer with 6 mm-diameter windows. All color measurements were obtained when the enamel surface was wet [17]. A VITA spectrophotometer was used for color assessment according to the CIE (Commission Internationale de l’Eclairage) LAB color systems, where $L^*$ represents the degree of lightness, $a^*$ represents the red/green axis, and $b^*$ represents the yellow/blue axis [18]. Before each measurement, the spectrophotometer was calibrated. The color of each tooth was measured three times, and the data were averaged. The color differences before and after bonding ($\Delta E^*$, $\Delta L^*$, $\Delta a^*$, and $\Delta b^*$) on days 7 and 28 were calculated using the following equations for color comparison [19]:

$$\Delta E^* = \left[ \left( \Delta L^* \right)^2 + \left( \Delta a^* \right)^2 + \left( \Delta b^* \right)^2 \right]^{1/2},$$

$$\Delta L^* = L^*_1 \text{ (or } L^*_2) - L^*_0,$$

$$\Delta a^* = a^*_1 \text{ (or } a^*_2) - a^*_0,$$

$$\Delta b^* = b^*_1 \text{ (or } b^*_2) - b^*_0,$$

where $L^*_0$, $a^*_0$, and $b^*_0$ are the baseline values, $L^*_1$, $a^*_1$, and $b^*_1$ are the values after debonding on day 7, and $L^*_2$, $a^*_2$, and $b^*_2$ are the values after debonding on day 28. When the $\Delta E^*$ value was higher than 3.7, it was considered unacceptable [20].

2.8. Statistical Analysis. SPSS version 22.0 (SPSS 22.0 for Windows, SPSS, Chicago, IL, USA) was used for statistical analysis. The Kolmogorov–Smirnov test and Levene’s test were used to determine the normality and homogeneity of the data, respectively [6]. Differences in the changes in the $L^*$, $a^*$, $b^*$, and $\Delta E^*$ values among all groups were compared using one-way ANOVA. Bonferroni’s test was performed for post hoc comparisons. The statistical significance level was 0.05.

3. Results

The baseline $L^*$, $a^*$, and $b^*$ values of all groups and those recorded on days 7 and 28 are listed in Tables 1 and 2. The $\Delta E^*$ values of all groups recorded on days 7 and 28 are listed in Table 3.

A significant decrease in the $L^*$ value was observed for all groups, indicating that the teeth darkened throughout the discoloration procedure (Table 2). There were no differences among the $\Delta L^*$ values of the RC, RD, BC, and BD groups. On day 7, significant differences were observed between the $\Delta L^*$ values of the RCTR and RC groups ($P = 0.001$), the RCTR and RD groups ($P = 0.039$), the BCTR and BC groups ($P = 0.009$), and the BCTR and BD groups ($P = 0.028$) (Table 2). After an immersion time of 28 days, the $\Delta L^*$ values of the RC and BC groups became significantly less than those of the RD and BD groups (Table 2). There were no significant differences between the $\Delta L^*$ values of the RCTR, RC, and RD groups and those of the corresponding BCTR, BC, and BD groups (Table 2). Significant differences were still observed between the $\Delta L^*$ values of the RCTR and RC groups ($P < 0.001$), the RCTR and RD groups ($P = 0.011$), the RC and RD groups ($P < 0.001$), the BCTR and BC groups ($P < 0.001$), the BCTR and BD groups ($P < 0.001$), and the BC and BD groups ($P = 0.001$) (Table 2).

A slight increase in the $\Delta a^*$ value was observed for all groups, indicating a shift toward red color components. The $\Delta a^*$ values of the control groups exhibited more marked changes than those of the RC, RD, BC, and BD groups on day 7 (Table 2). Significant differences were observed between the $\Delta a^*$ values of the RCTR and RC groups ($P = 0.004$), the RCTR and RD groups ($P = 0.028$), the BCTR and BC groups ($P = 0.001$), and the BCTR and BD groups ($P = 0.009$) (Table 2). There were no differences between the $\Delta a^*$ values of the RC and RD groups or those of the BC and BD groups (Table 2). On day 28, the color changes indicated by the differences in the $\Delta a^*$ values were similar for the control groups and the RD and BD groups (Table 2). The $\Delta a^*$ values of the RC and BC groups shifted the least (Table 2). Significant differences were observed between the $\Delta a^*$ values of the RCTR and RC groups ($P = 0.009$), the RC and RD groups ($P = 0.026$), the BCTR and BC groups ($P = 0.017$), and the BC and BD groups ($P = 0.002$) (Table 2). There were no differences between the $\Delta a^*$ values of the RCTR and RD groups or those of the BCTR and BD groups.

A significant increase in the $\Delta b^*$ value was observed for all groups, indicating a shift toward yellow color components. The $\Delta b^*$ values of the control groups showed significant changes throughout the study compared to those of the RC, RD, BC, and BD groups (Table 2). On day 7, no significant differences were observed between the $\Delta b^*$ values of the RC and RD groups or those of the BC and BD groups (Table 2).
ever, the Δ and the BCTR and BD groups (\(P^*\) significant differences between the different groups (Table 1). Significant differences were observed between the different groups (\(P < 0.05\)), whereas the same letter indicates no significant differences (\(P > 0.05\)).

### Table 1: Means and standard deviations (SDs) of the baseline \(L^*, a^*,\) and \(b^*\) values of each group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>(L^*) (Means ± SDs)</th>
<th>(a^*) (Means ± SDs)</th>
<th>(b^*) (Means ± SDs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCTR</td>
<td>83.64 ± 2.62(^A)</td>
<td>1.09 ± 1.28(^B)</td>
<td>29.51 ± 3.75(^C)</td>
</tr>
<tr>
<td>RC</td>
<td>84.24 ± 3.36(^A)</td>
<td>1.10 ± 1.24(^B)</td>
<td>29.33 ± 4.10(^C)</td>
</tr>
<tr>
<td>RD</td>
<td>84.73 ± 3.09(^A)</td>
<td>1.34 ± 1.17(^B)</td>
<td>31.06 ± 3.21(^C)</td>
</tr>
<tr>
<td>BCTR</td>
<td>84.10 ± 3.61(^A)</td>
<td>1.29 ± 1.23(^B)</td>
<td>30.38 ± 3.24(^C)</td>
</tr>
<tr>
<td>BC</td>
<td>83.22 ± 2.10(^A)</td>
<td>1.28 ± 1.07(^B)</td>
<td>29.39 ± 4.23(^C)</td>
</tr>
<tr>
<td>BD</td>
<td>86.13 ± 3.85(^A)</td>
<td>0.77 ± 1.22(^B)</td>
<td>29.24 ± 4.08(^C)</td>
</tr>
</tbody>
</table>

\(\Delta\) RCTR: resin control group; RC: resin Clinpro group; RD: resin Duraphat group; BCTR: bracket control group; BC: bracket Clinpro group; and BD: bracket Duraphat group. \(L^*\): the lightness value; \(a^*\): red/green axis value; \(b^*\): yellow/blue axis value.

Different uppercase letters in the same column indicate significant differences for the different groups on day 7 or 28 (\(P < 0.001\)). Different lowercase letters in the same row indicate significant differences among the different time points in the same groups (\(P < 0.05\)).

### Table 2: Comparison of the means and standard deviations (SDs) of the \(\Delta L^*, \Delta a^*,\) and \(\Delta b^*\) values of each group on days 7 and 28.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Means ± SDs (day 7)</th>
<th>Means ± SDs (day 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Delta L^*)</td>
<td>RCTR</td>
<td>-1.29 ± 0.70(^Aa)</td>
<td>-2.48 ± 0.33(^Ab)</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>-0.10 ± 1.05(^Bc)</td>
<td>-1.43 ± 0.31(^Bd)</td>
</tr>
<tr>
<td></td>
<td>RD</td>
<td>-0.56 ± 0.22(^Be)</td>
<td>-2.08 ± 0.46(^Cf)</td>
</tr>
<tr>
<td></td>
<td>BCTR</td>
<td>-1.30 ± 1.29(^Af)</td>
<td>-2.58 ± 0.45(^Ah)</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>-0.43 ± 0.50(^Bi)</td>
<td>-1.43 ± 0.24(^Bj)</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>-0.52 ± 0.21(^Bk)</td>
<td>-1.99 ± 0.27(^Cl)</td>
</tr>
<tr>
<td>(\Delta a^*)</td>
<td>RCTR</td>
<td>0.60 ± 0.59(^Ca)</td>
<td>0.96 ± 0.67(^Da)</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>0.19 ± 0.12(^Db)</td>
<td>0.43 ± 0.17(^Ec)</td>
</tr>
<tr>
<td></td>
<td>RD</td>
<td>0.29 ± 0.18(^Dd)</td>
<td>0.88 ± 0.69(^Ee)</td>
</tr>
<tr>
<td></td>
<td>BCTR</td>
<td>0.81 ± 0.20(^Ff)</td>
<td>0.99 ± 1.56(^Fe)</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>0.31 ± 0.32(^Dh)</td>
<td>0.52 ± 0.21(^Eh)</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>0.44 ± 0.13(^Di)</td>
<td>1.15 ± 0.22(^Dj)</td>
</tr>
<tr>
<td>(\Delta b^*)</td>
<td>RCTR</td>
<td>4.00 ± 2.55(^Fa)</td>
<td>6.98 ± 0.64(^Fh)</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>1.38 ± 1.88(^Fc)</td>
<td>2.37 ± 1.24(^Gc)</td>
</tr>
<tr>
<td></td>
<td>RD</td>
<td>2.28 ± 1.73(^Fd)</td>
<td>5.94 ± 0.65(^Hc)</td>
</tr>
<tr>
<td></td>
<td>BCTR</td>
<td>4.62 ± 1.41(^Ef)</td>
<td>7.01 ± 0.43(^Gf)</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>2.03 ± 0.54(^Fh)</td>
<td>4.01 ± 0.62(^Gi)</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>1.87 ± 1.62(^Fj)</td>
<td>5.89 ± 0.59(^Hk)</td>
</tr>
</tbody>
</table>

Different uppercase letters in the same column indicate significant differences for the different groups on day 7 or 28 (\(P < 0.001\)). Different lowercase letters in the same row indicate significant differences among the different time points in the same groups (\(P < 0.05\)).

### Table 3: Comparison of the means and standard deviations (SDs) of the \(\Delta E^*\) values of each group on days 7 and 28.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Means ± SDs (day 7)</th>
<th>Means ± SDs (day 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Delta E^*)</td>
<td>RCTR</td>
<td>4.90 ± 0.87(^Aa)</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>2.34 ± 0.85(^Bc)</td>
</tr>
<tr>
<td></td>
<td>RD</td>
<td>2.41 ± 1.09(^Dd)</td>
</tr>
<tr>
<td></td>
<td>BCTR</td>
<td>5.05 ± 1.30(^Fr)</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>2.00 ± 0.32(^Fh)</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>2.48 ± 0.53(^Fg)</td>
</tr>
</tbody>
</table>

Different uppercase letters in the same column indicate significant differences for the different groups on day 7 or 28 (\(P < 0.005\)). Different lowercase letters in the same row indicate significant differences among the different time points in the same groups (\(P < 0.05\)), whereas the same letter in a column or in a row indicates no significant differences (\(P > 0.05\)).

Significant differences were observed between the \(\Delta b^*\) values of the RCTR and RC groups (\(P = 0.001\)), the RCTR and RD groups (\(P = 0.024\)), the BCTR and BC groups (\(P = 0.001\)), and the BCTR and BD groups (\(P < 0.001\)) (Table 2). However, the \(\Delta b^*\) values of the RC and BC groups were significantly lower than those of the RD and BD groups on day 28 (Table 2). Significant differences were observed between the \(\Delta b^*\) values of the RCTR and RC groups (\(P < 0.001\)), the RCTR and RD groups (\(P = 0.002\)), the RC and RD groups (\(P < 0.001\)), the BCTR and BC groups (\(P < 0.001\)), the BCTR and BD groups (\(P < 0.001\)), and the BC and BD groups (\(P < 0.001\)) (Table 2).

On day 7, the \(\Delta E^*\) values of both the RCTR and BCTR groups were higher than 2.7, which is the clinical limit of detection by the naked eye (Table 3). On day 28, the \(\Delta E^*\) values of all groups except for the RC group were higher than 3.7 (Table 3), which was considered unacceptable. Significant differences were observed between the \(\Delta E^*\) values of the RCTR and RC groups (\(P < 0.001\)), the RCTR and RD groups (\(P < 0.001\)), the BCTR and BC groups (\(P < 0.001\)), and the BCTR and BD groups (\(P < 0.001\)) (Table 3). These differences increased as the experiment progressed over 28 days (all \(P < 0.001\)) (Table 3). There were no significant differences among the \(\Delta E^*\) values of the Clinpro™ XT and Duraphat groups on day 7 (Table 3). However, the \(\Delta E^*\) values of the Duraphat groups increased with increasing immersion time (Table 3). No significant differences were observed among...
the $\Delta E^*$ values of the resin and metal bracket groups on days 7 or 28 (Table 3).

4. Discussion

Information regarding the efficacy of Clinpro™ XT and Duraphat varnish in protecting teeth from discoloration is limited. The current study can be considered the first investigation in this field. Based on the findings of this study, the following null hypotheses were rejected: ① Clinpro™ XT and Duraphat varnish are not effective in preventing tooth discoloration during orthodontic treatment; ② Clinpro™ XT and Duraphat varnish do not show any significant differences in protecting teeth from discoloration during orthodontic treatment. However, the following null hypothesis was accepted: ③ there are no significant differences in tooth discoloration during orthodontic treatment between appliances attached with metal brackets and those attached with resin.

This study was performed in vitro not only for ethical reasons but also to standardize the study conditions to exclude differences in diet as a confounding factor and to facilitate identical testing conditions for Clinpro™ XT and Duraphat. Artificial saliva was also used to better reflect the oral environment due to its ability to deposit a pellicle layer on teeth [21]. Generally, tooth discoloration is influenced by not only food colorants or dietary pigments but also low pH media [22]. As the most popular beverage worldwide, coffee has the most potential for staining among common daily drinks because it is acidic and contains a variety of chemical compounds [23]. The coffee in this study had an acidic pH value, similar to that in previous studies [24, 25]. The low pH value of coffee might induce enamel dissolution and increase surface porosity, thus promoting tooth discoloration. The 28-day immersion time was chosen based on many previous and similar studies having a maximum immersion time of 30 days [26, 27]. Ertas et al. reported that 28 days were equivalent to approximately 2.5 years of clinical aging, which is approximately the duration of routine orthodontic treatment [28].

Usually, coffee causes an acidic and low-polarity stain via the demineralization of the enamel surface and the adsorption of coffee onto the tooth. Furthermore, demineralization changes the refractive index of enamel, altering its color properties [20]. Fluoride varnish forms a layer of calcium fluoride material on the enamel surface that can protect against acid attack. Thus, it inhibits the demineralization of the enamel surface [29]. The layer formed by fluoride varnish on the enamel surface can even act as a shield, reducing the adsorption of coffee [30]. According to our results, the Clinpro™ XT and Duraphat varnish groups both showed less discoloration than the control groups.

On day 7 of the study, no significant differences were found between the Clinpro™ XT and Duraphat groups, whereas these two groups exhibited significant differences on day 28. Due to the inherent difference in the fluoride carrier among commercially available fluoride varnishes, all fluoride varnishes release fluoride, but the method and duration of release vary [31]. Clinpro™ XT is a resin-modified glass ionomer cement that contains fluoroaluminoisilicate glass particles. The remineralization potential of this material is attributed to its manufacturing process, which creates functionalized calcium and free phosphate. The fluoride at the surface is released immediately, while the internal fluoride serves as a storage pool for sustained fluoride release during orthodontic treatment [11]. Shah et al. reported that a single application of Clinpro™ XT prevented enamel demineralization for up to 120 days, while the conventional fluoridated varnish prevented enamel demineralization for just 40 days; this difference was attributed to the functionalized calcium and free phosphate in Clinpro™ XT [13]. On the other hand, Duraphat is a varnish containing 5% sodium particles that adhere to the tooth surface after encountering saliva and detach after 24 hours, which differs from the behavior of light-curable varnish. Thus, due to the short retention time of Duraphat on the tooth surface, it needs to be applied frequently (every three months) to maintain its effectiveness [32]. The lower remineralization capacity of Duraphat leads to less protection and less tooth discoloration prevention.

In clinical situations, the corrosion of orthodontic alloys can occur, followed by the release of large amounts of nickel and chromium ions [9], resulting in tooth discoloration [1]. Götz et al. observed high metal ion levels after 2 weeks of metal appliance exposure [33]. Nevertheless, no differences were observed between metallic and nonmetallic braces in the present in situ experiment. This difference is most likely due to the variations in the conditions of metal corrosion. Mashallah et al. and Antonija et al. also reported the release of orthodontic metal ions from orthodontic metal brackets [34, 35], which was evaluated 6 months after the beginning of their studies. The limited experimental time of the present study may also have been a factor contributing to this difference. Another possible explanation could be that coffee has a much greater effect on tooth discoloration than metal alloys, thus preventing the observation of the effects of metal ions. Reduced or no friction between brackets and archwires might reduce the release of metal ions, which could also serve as a possible explanation for this difference [33].

In this study, we observed increases in both the $a^*$ and $b^*$ values with coffee exposure, suggesting that the color of the teeth became dark and shifted toward more reddish-yellowish colors, which should be related to the adsorption of coffee onto the enamel [7]. The direction of tooth color change was consistent with the color of the staining agent [4]. In this study, the changes in the $a^*$ and $b^*$ values of the fluoride varnish groups were significantly different from those of the control groups. Fluoride varnish not only acts as a barrier on the tooth surface, reducing the direct adsorption of coffee onto the enamel, but also prevents pigments from entering the internal structure of the tooth by facilitating remineralization in micropores, gaps between the glaze columns, and places with poor mineralization.

If the average orthodontic treatment duration is assumed to be 2 years, traditional fluoride varnish would need to be applied up to 24 times throughout the course of treatment to
achieve almost complete protection, whereas Clinpro™ XT would only need to be applied 4 to 6 times [36]. The frequency of Clinpro™ XT application would be nearly three times less, which is a considerable reduction that merits its recommendation for protecting teeth from discoloration.

Although the results of the study further the understanding of the potential differences in fluoride release characteristics, they must be interpreted with caution since the study period was limited. In vitro experimental conditions cannot simulate all complex factors affecting the human mouth in vivo, including aging and plaque, and the final results regarding the efficiency of these forms of fluoride varnish should be obtained through controlled clinical trials in the future. Additionally, the protective effects of these fluoride varnishes were evaluated without mechanical action, which can be considered another limitation of this study. Notably, a recent study showed that the use of a toothpaste containing hydroxyapatite could be a more reliable method for the domiciliary management of WSLs than the use of conventional fluoride toothpaste [37]. It might be interesting to compare the efficacy of toothpaste containing biomimetic hydroxyapatite with that of the two varnishes investigated in this study.

5. Conclusion

Within the limitations of this study, the following conclusions can be drawn:

(i) The application of both tested fluoride varnishes during orthodontic treatment demonstrated significant and beneficial effects in preventing tooth discoloration

(ii) Clinpro™ XT varnish was more effective in protecting teeth from discoloration than Duraphat varnish over time

(iii) No significant difference was observed in tooth discoloration between the appliances attached with metal brackets and those attached with resin.

Data Availability

The data supporting the findings of this article are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this study.

Authors’ Contributions

Lin Xu and Yuchun Zou contributed equally to this work and share first authorship. L.X., Y.C.Z., and L.Y.X. conceptualized the study; L.X., Y.C.Z., H.Y.L., and X.H.Y. carried out the investigation and curated the data; L.X. wrote the original draft; Y.C.Z. and L.Y.X. revised and made corrections to the manuscript. All authors have read and agreed to the final version of the manuscript.

Acknowledgments

The authors would like to thank Prof. Hao Yu (School and Hospital of Stomatology, Fujian Medical University) and his researchers, especially Hui Yang, for their advice on methodology. This work was supported by the Project of the Fujian Provincial Department of Finance in China (grant no. 2021CZ03 to LYX) and the Fujian Provincial Health Planning Commission Medical Innovation Project in China (grant no. 2021CXB017 to LYX).

Supplementary Materials

Supplemental Figure 1: study scheme. Supplemental Figure 2: study materials and methods. A: obtained human premolars (n = 140); B: extracted tooth retainer; C1: template of resin attachment; C2: 6 mm-aperture colorimetric tray; C3: 3.5 mm-aperture acid etching tray; D: storage in coffee for teeth in all groups. Supplemental Figure 3: template of resin attachment. A: a group of resin attachments; B: alginate impressions; C: white plaster model; D: area of resin attachment; E: resin attachment; F: template of resin attachment. Supplemental Figure 4: coffee cycling. (Supplementary Materials)

References

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