Assessment of Enamel Demineralization Adjacent to Orthodontic Brackets Using a Polarized Microscope

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Abstract

Background and Objectives: This study was conducted to assess the effect of light-curable fluoride varnish on enamel demineralization adjacent to orthodontic brackets using polarized light microscopy and to compare the depth of demineralization at different time periods.

Method: In 15 patients, the first premolars were allocated into 2 groups. In the experimental group light-curable fluoride varnish was applied. At the end of each time period (60, 90, and 120 days), first premolar brackets were debonded and premolars were extracted. Buccolingual sections were evaluated under a polarized light microscope and depth of demineralization was assessed.

Results: The depth of demineralization in the control group increased from 60 to 120 days, and the experimental group did not show any significant difference during the time period.

Conclusion: Single application of light-curable fluoride varnish, Clinpro XT can be effective in reducing enamel demineralization during fixed orthodontic mechanotherapy, especially in noncompliant patients.

Keywords
Clinpro XT, enamel demineralization, polarized microscope

Introduction

In fixed orthodontic treatment, the chance of occurrence of white spots is comparatively more and is a matter of concern. There are mainly two contributing factors for enamel demineralization, which include the complex structure of orthodontic brackets and etching of enamel for bonding orthodontic brackets. A white spot lesion is defined as “subsurface enamel porosity from carious demineralization,” which presents itself as an opaque milky white surface. During fixed appliance orthodontic therapy, dental plaque accumulation is more, and the pH of plaque is less compared to non-orthodontic patients, and levels of acidogenic bacteria like Streptococcus mutans and Lactobacillus are also increased.

As the pH of plaque drops below the threshold of remineralization, the process of demineralization starts to occur. The lesion starts with dissolution of hydroxyapatite crystals from the enamel prisms that form the superficial surface of the enamel. At a certain point of time, the demineralization appears clinically as white spot lesions. The periphery of orthodontic brackets becomes a responsive site for plaque retention; hence, there is an increased risk for enamel demineralization.¹ The occurrence of white spot lesions in patients receiving fixed appliance treatment is up to 50% and can be seen 1 month post starting orthodontic therapy when no preventive fluoride programs are used.

Once the lesion is established, it may lead to various subsurface lesions, which are difficult to be controlled. The teeth which are commonly affected by white spot lesion include maxillary lateral incisors and mandibular canine, even though there is a generalized distribution of white spot lesion, and the distogingival part of the labial surface is more prone to demineralization.² There are many methods to prevent enamel decalcification, which include maintaining proper oral hygiene by following proper brushing techniques, modifying the diet by reducing carbohydrate intake, and applying prophy powder, prophy paste, fluoride paste.³

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Varnishes provide a high fluoride concentration to prevent
demineralization. The adherence of varnish to teeth is more
compared to topical fluorides, which show increased ability
of enamel for fluoride uptake.\(^4\) Compared to other sources
of fluoride applications such as monofluoride phosphate
dentifrices, acid phosphate fluoride gel, and fluoride rinse,
fluoride varnish permits incorporation of more fluoride due
to prolonged contact time with the tooth surfaces. Vivaldi-
Rodrigues et al reported trimonthly application of fluoride
varnish after orthodontic treatment reduced the white spot
lesion incidence rate by around 44%. Teeth that had been
previously subjected to fluoride varnish around composite
resin-bonded orthodontic brackets showed 35% reduction in
the depth of the demineralized lesion.\(^5\)

Certain kinds of light-curable fluoride varnish remain
intact to the tooth surface for a longer duration compared to
regular fluoride varnish and can resist tooth abrasion caused
by brushing and wear for 6 months or more; moreover, it helps
in remineralization. The purpose of this study is to find out
the effectiveness of a single application of highly filled resin-
modified glass ionomer-based light-cure fluoride varnish on
effectiveness of demineralization around orthodontic brackets by using
polarized light microscopy.

Materials and Methods

Fifteen patients whose fixed orthodontic treatment involved
all first premolar extraction were recruited for the study.
The study was designed such that for each patient, the first
premolars were allocated to either the control group or the
experimental group. Total sample size is 15 patients (4
first premolars each patient)—control group 30 teeth and
experimental group 30 teeth.

The experimental group was treated with light-curable
fluoride varnish, Clinpro XT. (Figure 1). Non-randomized
split-mouth design was used, in which diagonally opposite
quadrant received the same treatment. Every alternate patient
received the same application pattern in the respective
quadrant.

Single blinding was done in this study. The patients were
not aware of the intervention being done.

Figure 1. Light-Curable Fluoride Varnish, Clinpro XT

Figure 2. Light-Curable Fluoride Varnish, Clinpro XT Was Applied
on the Buccal Surface and Light-Cured for 20 s

After cleaning the tooth with non-fluoridated pumice
paste, enamel surfaces were etched with 37% phosphoric acid
for 30 s, followed by rinsing and air drying. After this stage,
Transbond XT primer was applied on the etched enamel
surface. MBT 022 slot standard stainless steel brackets were
bonded with Transbond XT adhesive resin.

Experimental group—light-curable fluoride varnish,
Clinpro XT was applied on the buccal surface and cured for
20 s using a light cure unit (Figure 2).

All patients were instructed to maintain standard oral
hygiene, and non-fluoridated toothpaste was advised until the
end of each time period. After bonding, at the end of each time
period (60, 90, and 120 days), the first premolar brackets were
debonded, and premolars were extracted. A careful debonding
procedure was used to prevent enamel microfractures around
the bracket base. Extracted premolars in both experimental
and control groups were stored in normal saline solution.
Each tooth was then embedded in self-cure acrylic resin.

Buccolinguol sagional sections were made from the middle third
crown by using a rotating carborundum disc attached to a
micromotor, with continuous drip of water. The thickness of
each section was further reduced by hand grinding (Figure 3).

Sections were evaluated under a polarized light microscope
(Olympus CX41, Tokyo, Japan) (Figure 4). Microphotographs
of the buccal surface were taken with the fixed magnification
of 20 times (Figure 5). The depth of enamel demineralization
was assessed using Image J software.

Statistical Analysis

Statistical Package for Social Sciences [SPSS] for Windows
Version 22.0 (released 2013, IBM Corp., Armonk, NY) was
used to perform statistical analyses.

Descriptive Statistics

Descriptive analysis of all the explanatory parameters
was done using frequency and proportions for categorical
variables, and using Mean and SD for continuous variables.

Inferential Statistics

Wilcoxon signed rank test was used to compare the mean
depth of demineralization between experimental and control
groups at different time intervals.
Figure 3. The Thickness of Each Section Was Further Reduced by Hand Grinding

Figure 4. Polarized Light Microscope (OLYMPUS CX41, Tokyo, Japan)

Figure 5. Microphotograph of the Buccal Surface

Figure 6. Age Distribution Among Study Subjects

Figure 7. Gender Distribution Among Study Subjects

Figure 8. Comparison of Mean Depth of Enamel Demineralization (in µm) between experimental and control groups at different time intervals

Notes: The mean depths of demineralization in the control group are 52.818 µm, 86.106 µm, and 122.772 µm at time intervals of 60 days, 90 days, and 120 days, respectively. The mean depths of demineralization in the experimental group are 0.436 µm, 1.008 µm, and 1.212 µm at time intervals of 60 days, 90 days, and 120 days, respectively.
Results

This is an in vivo study to assess the effect of light-cureable fluoride varnish on enamel demineralization around orthodontic brackets bonded to first premolars by using polarized light microscopy. Fifteen patients fulfilling the inclusion criteria were included in the study.

Totally, 60 teeth were divided equally (refer Figures 6, 7 and Table 1 for age and gender distribution) into experimental and control groups based on 3 time intervals (60, 90, and 120 days). The objective of the study is to compare the depth of demineralization of enamel in the experimental (group in which fluoride varnish is applied) and control groups.

Discussion

The appearance of white spot lesion after fixed mechanotherapy is esthetically discouraging as the goal of orthodontics specialty is to improve dental and facial esthetics. Insertion of fixed appliances creates stagnation areas and, thus, alters the ecology, resulting in increased microbial content. The principal agent to reduce demineralization is fluoride. In this study, a light-cureable fluoride varnish, CLINPRO XT is used for the clinical trial. Clinpro XT varnish is a site-specific, resin-modified light-cured glass ionomer durable cement coating that forms an immediate layer of protection to prevent enamel demineralization. The “XT” in Clinpro XT represents “extended varnish” having durability, with sustained release of fluoride. Clinpro XT releases more fluoride compared to conventional fluorides, and they are more resistant to brushing strokes, approximately 5000 strokes. This resistance is due to the higher filler content of the resin-modified light-cureable varnish.

The effect of light-cureable fluoride varnish on enamel demineralization was studied for a period of 4 months at time intervals of 60 days, 90 days, and 120 days without interfering with the regular treatment process. The depth of demineralization was assessed by using polarized light microscopy technique. In the control group, demineralization depth increased from 60 to 120 days; (Figure 9, Tables 4 and 5) however, symptoms of dentinal hypersensitivity were not reported during the study except for 2 patients in the 120-day group (Figure 9). In the experimental group, there is no statistically significant difference in lesion depth at different time intervals (Figure 10 & Table 3). There are only limited in vivo studies related to the use of light-cureable fluoride varnish to assess the depth of demineralization for a period of 4 months. The closest in vivo study was one by A. Mehta et al to evaluate the depth of demineralization, and they concluded that the mean lesion depth is 10.6 µm in the experimental group for a period of 90 days compared to 1.008 µm in the present study.
Effectiveness of Conventional Fluoride Varnish Application

Du et al evaluated the effectiveness of Duraphat fluoride varnish for a period of 3 and 6 months. The assessment was carried out using DIAGNOdent, and it was found that Duraphat fluoride varnish was effective in the reversal of white spot lesions. In a recent split-mouth study by Perrini et al, when applied at time intervals of 3, 6, 9, and 12 months, Duraphat did not show a significant difference in enamel demineralization in control and experimental groups. Repeated application of conventional fluoride varnish is recommended to prevent the occurrence of white spot lesions (WSL). Various regimens of application of fluoride varnish have been evaluated till date. Van Eck et al suggested one application per year; Beltran et al advocate semiannual application of fluoride varnish. In another study by Modeer et al, it was found that 4 applications of Duraphat fluoride varnish per year can reduce proximal caries progression. Monthly application of fluoride varnish was suggested by Schmit et al.

Primarily, varnishes used in dentistry contain biocompatible solvents, such as water, ethanol, acetone, or esters. In these solvents, film formers based on high-molecular polymers (eg, polyamides, cellulose derivatives) or low-molecular resins (eg, colophony) are dissolved. Once applied to the tooth, the solvent starts to evaporate, meaning the film former and active ingredients are available in ever-increasing concentrations. The increasing concentration of the solid and dissolved components of the varnish during evaporation of the solvent leads to closer proximity of the film-forming molecules to each other. This induces an increase in Van der Waals forces and the purely mechanical interactions of the molecules among each other. These interactions eventually lead to the immobility of the molecules and, thus, the formation of a calcium fluoride layer on the tooth surface, which resists subsequent enamel demineralization.

Effectiveness of Light-Curable Fluoride Varnish

Liquid varnishes are based on established dental methacrylates (eg, HEMA, bis GMA). They are diluted to an easy-flowing consistency with solvents (eg, methyl methacrylates, dipentaerythrol pentaacrylates). The addition of blue light-sensitive photoinitiators enables the quick formation of a film when radical polymerization takes place after a dental curing light is illuminated. The most prevalent type of photoinitiators are carbonyl compounds such as diketones or other acrylic components.

A chain reaction in which the radicals formed by the photoinitiator react with the methacrylate monomers results in giving rise to a polymer network that envelops the active ingredients and, thus, permits their extended release. Clinpro XT, light-curable fluoride varnish, provides a rapid release of fluoride during initial few days and, thereafter, sustained release. The fluoroalumosilicate glass particles present in the surface of varnish provide the immediate release of fluoride by certain surface reactions, while interior provides the reservoir for sustained release of fluoride.

Kumar Jena et al conducted a study using resin-modified glass ionomer cement varnish for a period of 6 months, and demineralization was assessed by DIAGNOdent. The results were promising as light-curable fluoride varnish was effective, but careful monitoring was essential for DIAGNOdent reading, as it is susceptible to various local environmental factors such as plaque, calculus, and stains. The present study focused on histological sectioning and assessment of enamel demineralization using polarized light microscopy, and hence is more dependable than other studies.

Conclusion

Based on the results obtained in our study, the following conclusions may be drawn. There was an increase in depth of demineralization of enamel lesions from 60 to 120 days. In the experimental group, there is no statistically significant difference between mean lesion depth during different time periods. Statistically significant difference in mean lesion depth was noted between the experimental and control groups at the end of 60 days, 90 days, and 120 days (Figure 8 & Table 2). A single dose of light-curable fluoride varnish, Clinpro XT is effective in preventing enamel demineralization adjacent to orthodontic brackets. Light curable fluoride varnish (LCFV) is beneficial in high-risk and noncompliant patients and can be considered as the best fluoride delivery regimen. This study concluded that single application of light-curable fluoride varnish, Clinpro XT is effective in reducing demineralization up to 4 months and is effective in high-risk and noncompliant patients.

Table 1. Age and Gender Distribution Among Study Subjects

| Variables | Categories | N  | %   |
|------------|------------|----|-----|
| Age        | 13–15 years| 5  | 33.3|
|            | 16–20 years| 8  | 53.3|
|            | >20 years   | 2  | 13.4|
| Gender     | Males      | 6  | 40  |
|            | Females    | 9  | 60  |

Note: In this study, age ranges from 13 years to 22 years with a mean age of 17.13 years and a standard deviation of +/- 2.64 were considered.
### Table 2. Comparison of Mean Depth of Enamel Demineralization Between Experimental and Control Groups at Different Time Intervals Using Wilcoxon Signed Rank Test

| Time    | Group    | N  | Mean  | SD    | Mean Diff | Z     | P-value |
|---------|----------|----|-------|-------|-----------|-------|---------|
| 60 days | Experimental | 5  | 0.436 | 0.305 | -52.382   | -8.132| .001*   |
|         | Control   | 5  | 52.818| 14.618|           |       |         |
| 90 days | Experimental | 5  | 1.008 | 0.305 | -85.098   | -29.401| <.001*  |
|         | Control   | 5  | 86.106| 6.715 |           |       |         |
| 120 days| Experimental | 5  | 1.212 | 0.419 | -121.560  | -13.798| <.001*  |
|         | Control   | 5  | 122.772| 19.725|           |       |         |

Notes: * Statistically significant. According to Wilcoxon signed rank test, a P-value less than .05 is considered as significant. Based on the P values, in the table, a statistically significant difference exists between the depth of enamel demineralization during comparison between the experimental and control groups. The mean differences in lesion depth between the experimental and control groups are 52.382, 85.098, and 121.560 during time intervals of 60 days, 90 days, and 120 days, respectively.

### Table 3. Comparison of Mean Depth of Demineralization Between Different Time Intervals in the Experimental Group Using Friedman Test

| Time    | N  | Mean  | SD    | Min | Max | FM  | χ² | P-value |
|---------|----|-------|-------|-----|-----|-----|-----|---------|
| 60 days | 5  | 0.436 | 0.305 | 0.10| 0.80| 4.105|     | .13     |
| 90 days | 5  | 1.008 | 0.305 | 0.50| 1.30|     |     |         |
| 120 days| 5  | 1.212 | 0.419 | 0.60| 1.50|     |     |         |

Notes: The P values in the table indicate no statistically significant difference exists between the depth of demineralization in the experimental group during different time intervals. The standard deviations are 0.305, 0.305, and 0.419 at time intervals of 60 days, 90 days, and 120 days, respectively.

### Table 4. Comparison of Mean Depth of Demineralization (in µm) Between Different Time Intervals in the Control Group Using Friedman Test

| Time    | N  | Mean  | SD    | Min  | Max | FM  | χ² | P-value |
|---------|----|-------|-------|------|-----|-----|-----|---------|
| 60 days | 5  | 52.818| 14.618| 33.60| 73.10| 10.000|     | .007*  |
| 90 days | 5  | 86.106| 6.715 | 76.90| 93.80|     |     |         |
| 120 days| 5  | 122.772| 19.725| 94.10| 140.20|     |     |         |

Notes: * Statistically significant. The P values in the table indicate statistically significant difference exists between the depth of demineralization in the control group at different time intervals of 60, 90, and 120 days, respectively. The standard deviations are 14.618, 6.715, and 19.725 at different time intervals of 60, 90, and 120 days, respectively.

### Table 5. Multiple Comparison of Mean Difference in Depth of Demineralization Between Time Intervals in the Control Group Using Wilcoxon Signed Rank Test

| (I) Time | (J) Time | Mean Diff. (I – J) | Lower | Upper | 95% CI for Diff. |
|----------|----------|--------------------|-------|-------|------------------|
| 60 days  | 90 days  | -33.288            | -66.570| 0.006 | .04*             |
| 60 days  | 120 days | -69.954            | -128.385| -11.523| .03*             |
| 90 days  | 120 days | -36.666            | -68.529| 4.803 | .03*             |

Notes: * Statistically significant. The P values in the table indicate statistically significant difference exists between the depths of demineralization during multiple comparisons in the control group. The mean difference in depth of demineralization is 33.288, when comparing the time intervals of 60 days and 90 days. The mean difference between 60 days and 120 days is 69.954, which is statistically significant. Statistically significant differences exist between time intervals of 90 days and 120 days with a mean difference of 36.666.
### Declaration of Conflicting Interests
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding
The authors received no financial support for the research, authorship, and/or publication of this article.

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