

Evaluating the Surface Microhardness of Four Types of Remineralizing Agents on The Surface of Enamel During Orthodontic Treatment

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ABSTRACT

An in-vitro study was designed to evaluate the surface micro-hardness (SMH) of four types of remineralizing agents on the surface of enamel during orthodontic treatment.

Aim

The aim of this laboratory study is to evaluate the surface micro-hardness (SMH) and the depth of demineralization of Amflor toothpaste, Amflor mouthrinse, MI fluoride varnish and Ormco bonding agents on enamel surface during orthodontic treatment.

Materials and Methods

To carry this out, researchers used 44 premolars that had been extracted for therapeutic reasons. After thorough cleaning, the teeth were stored in saline, mounted in acrylic resin, and selectively coated to leave a small testing area exposed. These samples were then divided into four groups based on the product being tested and then colour coded. Over a 15-day period, each group received regular applications of its respective agent, followed by storage in artificial saliva to mimic oral conditions.

At the end of the treatment phase, the teeth were examined for early signs of decay (white spot lesions), and a Vickers hardness test was performed to measure the enamel's surface strength. The study aims to shed light on which agent offers the best defence against enamel weakening during orthodontic care.

Results

Enhanced and highest mean SMH was present in MI fluoride varnish group followed by Amflor toothpaste group, Amflor mouthrinse group and Ormco bonding agent group respectively after application of remineralizing agents. Statistically significant differences were noted between group orange and red ($p < .0001$) except for all the others groups where significant differences weren't noted.

Conclusion

All the four agents employed in this study - Amflor toothpaste, Amflor mouthrinse, MI fluoride varnish and Ormco bonding agent improved the SMH of teeth after the therapy given over 15 days compared to the SMH following demineralization.

Keywords

Amflor, MI Fluoride varnish, Ormco bonding agent, Remineralization, White spot lesion, demineralization, surface microhardness (SMH)

INTRODUCTION

White spot lesions (WSL) are early signs of enamel damage, often appearing as chalky white patches on the tooth surface. They develop when minerals are lost from beneath the enamel, usually due to plaque build-up and prolonged bacterial activity that triggers decalcification over time.^[1]

White spot lesions are often the earliest warning signs before full-blown enamel cavities develop. In orthodontic patients, these spots typically form around

brackets and wires where plaque tends to build up and stay on the enamel surface for extended periods.^[2]

Even when using fluoride toothpaste, studies show that 13% to 75% of orthodontic patients with fixed braces can still develop enamel lesions. This wide range highlights how common and variable the risk can be during treatment.^[3] Fixed orthodontic appliances can make it harder to maintain oral hygiene, often leading to enamel mineral loss - commonly seen as white spot lesions on the teeth. ^[4]

Fixed braces, including brackets, bands, and wires, tend to trap plaque due to their uneven surfaces, making it easier for build up to occur around them. ^[5] Mutans Streptococci are key bacteria involved in the formation of white spot lesions, as they contribute significantly to enamel demineralization.^[6]

Among the various fluoride delivery systems, including gels, varnishes, toothpastes, and mouthrinse, fluoride varnishes offer distinct advantages due to their ease of application and reduced dependence on patient adherence. Fluoride varnish forms a thin, adherent layer on the enamel surface, enabling prolonged fluoride release and sustained therapeutic effect.^[13]

Consistent daily use of high-fluoride toothpaste has been shown to significantly reduce both the prevalence and development of enamel lesions adjacent to fixed orthodontic appliances. Amflor toothpaste, which primarily contains amine fluoride, an organic form of fluoride, supports the remineralization process by enhancing the repair of early enamel damage. This action helps to strengthen the enamel surface, making it more resistant to acid attacks commonly experienced during orthodontic treatment.^[7]

An array of techniques has been suggested to prevent the occurrence and severity of WSLs. Various strategies have been recommended to prevent the formation and progression of white spot lesions during orthodontic treatment. This study focuses on evaluating the effectiveness of multiple remineralization methods using an in vitro orthodontic retention model. Enamel samples are subjected to controlled conditions that simulate the oral environment, followed by assessments using optical evaluation techniques to observe lesion development. Additionally, changes in the SMH of enamel are quantified with a Vickers hardness testing machine, providing objective data on the protective and restorative potential of each remineralizing agent. The null hypothesis of the surface microhardness between four different remineralizing agents before and after placement in a demineralizing solution would not differ between the four groups.

MATERIALS AND METHODS

Armamentarium

A. Materials used

1. Extracted premolars(n=44)
2. Cold cure acrylic
3. Amflor mouthrinse (Group Pharma LATD, Mumbai, India)
4. Amflor toothpaste (Group Pharma LATD, Mumbai, India)
5. MI Fluoride varnish
6. Bonding agent (Orthosolo,Ormco, California, USA)
7. Nail Varnish
8. Artificial saliva
9. Saline solution
10. 3% hydrogen peroxide
11. 0.1% Thymol solution
12. Applicator tips



Figure 1: Amflor toothpaste



Figure 2: Amflor mouthrinse



Figure 3: MI Fluoride Varnish



Figure 4: Ormco Bonding agent

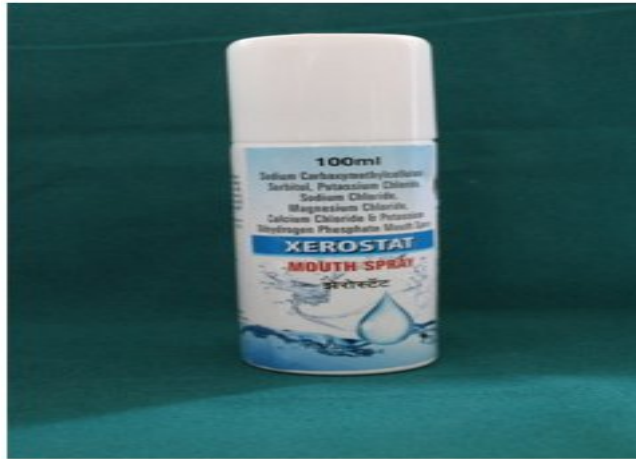


Figure 5: Artificial Saliva

B. Equipment Used

1. Vickers hardness testing machine



Figure 12: Vickers hardness testing machine

METHODOLOGY

The present in vitro study was conducted in the Department of orthodontics and Dentofacial Orthopaedics, HKE Society's Nijalingappa Institute of Dental Sciences, Karnataka, India.

Source of the data

Extracted premolars for the study were collected from orthodontic patients indicated with premolar extraction treatment plan for the purpose of fixed orthodontic mechanotherapy in the Department of Oral and Maxillofacial surgery, HKES's S Nijalingappa Institute of Dental Sciences and Research Kalaburagi.

Study Design: IN -VITRO

Total sample size -44 extracted teeth

Inclusion Criteria

- Extracted teeth with intact enamel from orthodontic patients indicated for extraction
- Non carious
- Non hypoplastic, without cracks and restoration

Exclusion Criteria

- Carious
- Non therapeutically extracted teeth
- Teeth with any developmental anomalies

Method of Collection of Data

A total of 44 extracted premolars of patients indicated with extraction treatment plan for fixed orthodontic mechanotherapy were collected and divided into four groups containing 11 in each group. Though molars

have a larger surface area it is difficult to get sound tooth and hence the premolars were selected.

The extracted teeth were cleansed with 3% hydrogen peroxide and normal saline and stored in 0.1%Thymol solution in a sealed container. At the time of conducting the research, the selected teeth were washed to remove the existing film and dried. The handpicked teeth samples were divided randomly into 4 groups(11 samples in each group) of different remineralizing agents .

The samples were painted with nail varnish not including a 3x3 mm (about 0.12 in) window in the middle of buccal surfaces of premolars and stored in saline. They were then coated with different remineralizing agents for every 12 hours for 5 minutes and then wiped off and placed in artificial saliva for 15 days(about 2 weeks)

The tooth samples are removed and mounted in acrylic resin and evaluated for white spot lesions and grading was done. Using the Vickers hardness testing machine the surface microhardness was estimated over three assorted points chosen on the premolar for a fixed load of 25N for 20 seconds. The mounted samples were divided into four groups and colour coded as shown below.

Grouping

Group 1-(Blue) Amflor toothpaste(n=11)

Group 2(Green)Amflor mouthwash(n=11)

Group 3(Red)MI Fluoride Varnish(n=11)

Group 4(Orange)Ormco Bonding Agent(n=11)



Figure 7: Application of Amflor mouthrinse

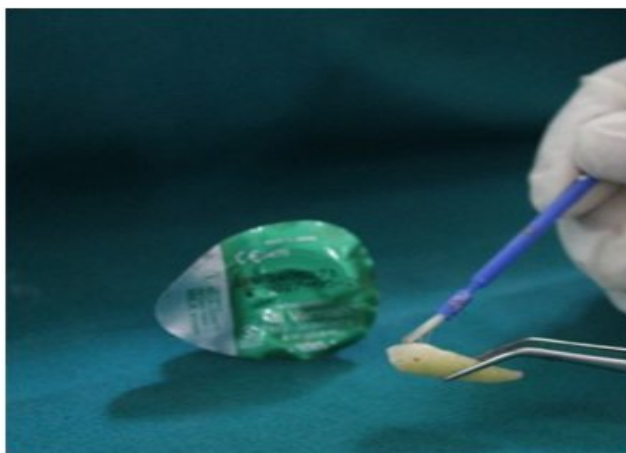


Figure 8: Application of MI fluoride varnish



Figure 9: Application of Amflor Toothpaste



Figure 10: Application of Ormco Bonding agent

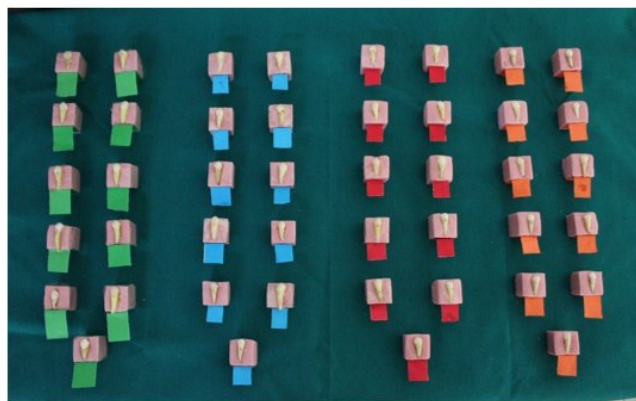


Figure 11: Colour coding of four different groups



Figure 14: Vickers hardness done with 25N load



Figure 13: Tooth sample subjected to Vickers hardness testing

RESULTS

Enamel demineralization after orthodontic treatment is a frequent concern for both dental professionals and their patients.

One of the most objectionable side effects of multiple bracket orthodontic therapy is the formation of WSLs on debonding. The elimination of these WSLs is a key challenge in enhancing aesthetics. The role of fluorides in the prevention of WSLs is well documented as fluoride ions alter the metabolism of bacteria in plaque by inhibiting enzymatic processes, production of acids, and reducing demineralization through promoting remineralization in initial stages especially at low concentrations.^[38]

To evaluate and utilize the property of fluorides, this in vitro study was undertaken to assess the surface micro hardness of enamel using four commercially available remineralizing agents after induction of WSLs by demineralization. These agents include Amflor toothpaste, Amflor mouthrinse, MI fluoride varnish, Ormco Bonding agent. Amflor toothpaste contains Amine Fluoride of 3.85% as its active fluoride ingredient. It provides a maximum of 1000 ppm (parts per million) of available fluoride when packed.

Amflor Mouthrinse contains Amine Fluoride as its active ingredient. It provides 480 ppm (parts per million) of available fluoride when packed.

GC MI Varnish (often just referred to as MI Varnish) contains 5% Sodium Fluoride (NaF). It contains 5% Sodium Fluoride is equivalent to 22,600 ppm fluoride ion. It's also important to note that MI Varnish is enhanced with RECALDENT™ (CPP-ACP), which stands for Casein Phosphopeptide-Amorphous Calcium Phosphate.^[39]

Ormco Ortho Solo is described as a fluoride-releasing universal sealant and bonding primer. It contains 200-1000ppm of fluoride with alkali fluorosilicates (eg: disodium hexafluorosilicate) with 0.1 -1%. The buccal surfaces of the tooth had been selected to test as brackets are bonded on the buccal surface of the tooth. Artificial saliva has been used to mimic the oral environment and to allow for WSL formation on the enamel surfaces respectively. Despite having different primary ingredients, all these agents have shown positive outcomes in promoting enamel remineralization.

Vickers hardness testing is a common method used in in- vitro studies to assess changes in enamel surface

microhardness(SMH) which is an indicator of mineral content and resistance to formation of carious lesions .A higher Vickers hardness number(VHN) generally indicates harder, more mineralized enamel while a lower VHN suggests demineralization or softer enamel. The relationship between white spot lesion (WSLs) and Vickers hardness is direct and inverse. White spot lesions are characterized by a decrease in enamel surface microhardness (SMH) when measured with a Vickers hardness test. A lower VHN directly correlates with the extent of demineralization in a white spot lesion.

The null hypothesis of the surface microhardness between four different remineralizing agents before and after application of demineralizing agents is different between four groups but rejected.

In the present study our results indicated that the SMH of teeth in all groups was higher after the therapy given over 15 days compared to microhardness following demineralization .Gorelicks’s grading system has been done to evaluate the white spot lesions of enamel.

Grading	Extent of white spot lesion
0	No WSL
1	Mild
2	Moderate
3	Severe

The test results suggests that the proportion of Gorelick grade 0 versus grade 1 does not show significantly differ across Blue, Green, Orange and Red groups.

In the study conducted using Vickers hardness testing machine to evaluate the surface microhardness of enamel showed that using the One way ANOVA indicated a significant difference across the four groups, indicating at least one coloured groups is significant from the others with a ($p < .0001$) The results indicated that the Blue group had a significantly higher Vickers hardness value than the orange group (mean difference = 9.27, $p = .0276$). The Green group had a significantly lower Vickers hardness value than the red group (mean difference = -9.36, $p = .0257$). A significant difference was also observed between the orange and red groups (mean difference = -16.55, $p < .0001$). Comparisons among the other group pairs—Blue vs. Green, Blue vs. Red, and Green vs. Orange—did not yield statistically significant differences.

The coefficient of significance was much higher between groups orange and red and almost similar between blue -orange and green- red groups. The higher Vickers hardness number shows that the surface microhardness of enamel is much higher while using MI fluoride varnish when compared to using Ormco bonding agent and Amflor toothpaste and Amflor mouthrinse. The amount of white spot lesion formed were least in the fluoride varnish group while it was highest in the Ormco bonding agent group and almost similar with mild difference between the Amflor toothpaste and Amflor mouthrinse groups. The fluoride ions in the oral cavity causes precipitation of fluorapatite from the calcium and phosphate ions in the saliva. The increased pH will lead to the formation of larger acid-resistant crystals containing fluoride (Flurohydroxyapatite)

The null hypothesis of using four different groups treated with different demineralizing agents would differ and it is rejected.

The current study found that the highest record difference in the value of surface hardness was for MI fluoride varnish followed by Amflor toothpaste. The least increase in microhardness was noted for the Ormco bonding agent group. This is also in accordance in study with Nalawade.^[40] where three agents employed in that study as Colgate sensitive pro-relief toothpaste, Amflor and Enafix improved the surface microhardness of teeth after 2 weeks compared to the microhardness following demineralization. The Amflor group showed least increase in microhardness. According to study conducted by Kakkar et al in concordance with the current study showed that both Enafix and Amflor are economic means to avoid demineralization of enamel. In his study Amflor depicted greater surface microhardness (SMH) than Enafix with the findings of the current study. In comparison to the study conducted by Trimpeneers and Dermaut which use a fluoride releasing bonding adhesive with a non fluoride releasing one and showed no significant difference between the decalcification rates for both the adhesives.^[41]

According to the study by Farshad Rahimi and Masoud Sadeghi showed that fluoride varnish combined with CHX (Chlorhexidine) could be effective. Treatment to reduce white spot lesions after orthodontic treatment.^[12]

In the study by Kiana Slehzadeh and Romina Mazaheri showed that one month use repeated application of all agents improved microhardness, the remineralization potential of CPP-ACP was

significantly higher than that of Remin Pro and Fluoride Varnish in concordance with the current study. In our knowledge this is the first study that has been performed to compare the remineralization potential of three easily available and economic fluoride containing agents that is Amflor toothpaste, Amflor mouthrinse, MI fluoride varnish and Ormco Bonding agent.

CONCLUSION

Within the experimental conditions and limitations of this study, the following conclusions were drawn.

1. Demineralization was observed in all four groups treated with different medicaments although they of a variable degree.
2. Although except for MI fluoride varnish and Ormco bonding agent, the other groups were not found statistically significant, they also prevent the enamel demineralization and are also commercially available and cost effective

All the four agents employed in this study that is Amflor toothpaste, Amflor mouthrinse, MI fluoride varnish and Ormco bonding agent after being subjected to Vickers hardness testing has been showed to have improved the surface microhardness of teeth after therapy given over 15 days compared to the microhardness following demineralization. We found that the highest record value of surface hardness was for MI fluoride varnish group followed by Amflor toothpaste group. The least increase in microhardness was noted for the Ormco bonding agent group.

Visible white spot lesions were observed in the Ormco bonding agent group and mild to no white spot lesions observed in the MI fluoride varnish group. This was followed by Amflor toothpaste and Amflor mouthrinse.

The present study demonstrated that MI fluoride varnish significantly enhanced the surface microhardness of enamel, as reflected by higher Vickers hardness Number (VHN) and was associated with the least incidence of white spot lesions among the groups tested. In contrast, the Ormco bonding agent exhibited the lowest enamel hardness values and highest susceptibility to WSL formation, indicating inferior protective effects against demineralization. These findings suggest that fluoride releasing varnishes, such as MI varnish, provides a superior protective benefit to enamel surfaces during orthodontic treatment, potentially mimicking the risk of enamel demineralization and enhancing long term enamel integrity.

Further studies with larger sample sizes, longer observation periods, and in vivo conditions are recommended to validate the long-term effectiveness of fluoride varnishes in preventing enamel demineralization during orthodontic treatment

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