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ORIGINAL RESEARCH

Evaluation of the Efficacy of Various Topical Fluorides on Enamel Demineralization Adjacent to Orthodontic Brackets: An *In Vitro* Study

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ABSTRACT

Aim: White spot lesions (WSLs) occur frequently after fixed orthodontic treatment. This *in vitro* study was undertaken to compare the efficacy of 2.26% fluoride varnish, 1.23% APF gel, 0.21% fluoride toothpaste and 0.04% sodium fluoride mouthwashes in preventing enamel demineralization around orthodontic brackets in extracted premolars.

Materials and methods: The sample for this study included 100 premolars free of caries and enamel cracks. They were divided into five groups of 20 samples each. Group 1 (FV): light-curable Fluoride varnish (Clinpro™ XT 3M ESPE, Pymble, New South Wales, Australia), group 2 (FG): 1.23% APF gel (Patterson NE. International, USA), group 3 (FTP): 0.21% w/w sodium fluoride toothpaste with tri-calcium phosphate (Clinpro™ Tooth Crème, 3M ESPE, Australia), group 4 (FMW): sodium fluoride 0.044% (w/v) mouthwash (Colgate® Phos-Flur® Ortho Defense Rinse, Colgate-Palmolive, NY) and group 5 (C): control. The samples were subjected to laboratory pH cycling. The demineralization changes in the enamel were assessed before the start of the experiment and after 14 days.

Results: There was a significant change in the mean Diagnodent score value (p <0.001) in all groups from day 1–day 14. The mean values were significantly different among groups at day 1 (p = 0.002), day 14 (p = 0.001) and also the change from Day 1 to Day 14 was significantly different among Groups (p = 0.001). The least change in the mean value from baseline to 14 days was seen in group 1 (FV) followed by group 3 (FTP), group 2 (FG), and group 4 (FMW) and then the group 5 (C).

Conclusion: All the topical fluorides tested were able to reduce the demineralization when compared to the control group under similar testing conditions, but to varying degrees. light-curable fluoride varnish outperformed all the topical fluorides followed by 0.21% w/w dodium fluoride toothpaste with tri-calcium phosphate, 1.23% Acidulated phosphate fluoride gel and sodium fluoride 0.044% (w/v) mouthwash. The control group where no topical fluoride was applied showed the least resistance to demineralization.

Clinical significance: Within the limitations of this study, routine application of light cured fluoride varnish (Clinpro) can be recommended to prevent enamel demineralization to prevent white spot lesions in patients receiving orthodontic treatment.

Keywords: Demineralization, Diagnodent®, Fluoride gel, Fluoride mouthwash, Fluoride toothpaste, Fluoride varnish, Remineralization, Topical fluoride, White spot lesion.

Keywords: Artificial teeth high impact acrylic resin, Debonding, Surface properties, Surface treatment.

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INTRODUCTION

Orthodontic treatment markedly improves the patient's esthetics, correct the malocclusions and significantly boost the self-confidence of the patients. However, orthodontic patients mostly comprised of the adolescent population, a group generally defined by overall non-compliance in maintaining oral hygiene. The presence of brackets, archwires, ligatures, and other orthodontic appliances further increase the retention of biofilm leading to demineralization around brackets in four weeks and encourage the formationof white spot lesions (WSL).[1]

White spot lesions (WSLs) are areas of subsurface enamel porosity resulting from caries demineralization, presenting as milky white opacity when present on smooth surfaces.

This process is potentially reversible, but irreversible once progress. Clinically detectable WSLs can occur as early as 4 weeks into fixed appliance treatment, and its prevalence among orthodontic patients ranges from 2–96%.

Several strategies can be employed to reduce the occurrence of WSLs,[2-4] including more frequent professional cleanings and reinforcing oral hygiene instructions by using applied behavior analysis (ABA), use of chemotherapeutic agents and other methods such as placement of sealants, fluoride varnishes on facial surfaces of the teeth, but preliminary research has shown conflicting results on their effectiveness.[5,6] Therefore, the present study was undertaken to compare the efficiency of 2.26% fluoride varnish, 1.23% APF gel, 0.21% Fluoride toothpaste and 0.04% NaF mouthwashes in preventing enamel demineralization around orthodontic brackets, when subjected to demineralization and remineralization challenge. These demineralization changes were measured using the Diagnodent instrument (KaVo, Germany).

MATERIALS AND METHODS

The sample of this in vitro study consisted of one hundred extracted premolar teeth which were collected from the Department of Oral and Maxillofacial Surgery, from orthodontic patients, requiring therapeutic extraction of premolars. The intact teeth free fromcaries and having no restorations were selected. Grossly deformed tooth, Hypocalcified tooth, Fluorotic tooth, Micro or Macrodontia, Teeth showing a Diagnodent score more than 10 were excluded from the study.

After extraction, the teeth were cleaned of residual soft tissue debris under running water with a toothbrush. The teeth were then stored in distilled water. This water was replaced every 48 hours to prevent bacterial contamination. A total of 100 extracted teeth were randomly divided into five groups using simple randomized sampling. The groups were as follows:

- *Group 1*: Fluoride varnish group (FV)
- *Group 2*: Acidulated phosphate fluoride gel group (FG)
- *Group 3*: Fluoride toothpaste group (FTP)
- *Group 4*: Fluoride mouthwash group (FMW)
- *Group 5*: Control group (C)

The buccal surface of each tooth was cleaned with a rubber cup and bristle brush on contra-angle handpiece with slow speed for 5 seconds. They were then washed, dried and etched with 35% phosphoric acid (Scotchbond™ Universal Etchant, 3M Unitek) for 30 seconds. After etching and drying, Transbond XT light cure adhesive primer (3M Unitek) was applied gingivo-occlusally for all the groups, followed by application of 3M Transbond XT Light cure adhesive to the bonding base of the brackets. The brackets were placed on the tooth surface lightly with the help of a bracket holding forceps, the final position was adjusted, and it was pressed firmly. Excess material was removed with an explorer and the adhesive was cured with a Light Emitting Diode (Ledition, Dentsply India). Curing was done for 20 seconds on each side i.e. mesially and distally. All the procedures were done by a single operator to avoid interoperator variability.

Each sample tooth was painted with an acid-resistant varnish leaving a window of 3mm length × 4mm width from the cervical area of bracket margin as measured by a ruler so that most of the crown was covered by acid-resistant varnish and only the exposed enamel could be attacked by acid. For ease of identification, five different colors of acid resistant varnishes were used. All the samples were examined using DIAGNOdent® (KaVo, Biberach, Germany) to assess for any surface changes present on the labial window. In this study, type B probe is used. As recommended by the manufacturer, before every measurement session, the instrument was calibrated against its ceramic disc standards. The labial window area was carefully scanned using the type B probe by holding the tip in close contact with the tooth surface and tilting the tip around the measuring area to collect the fluorescence from all directions. Samples showing a moment value between 0 and 10 on the digital display were selected. The baseline values of the five groups were then recorded.

Individual Group Treatment

- *Group I–Fluoride varnish (FV):* The teeth in this group were acid-etched for 15 seconds in the exposed enamel window, and a layer of Clinpro™ XT Varnish was applied and cured for 20 seconds with the curing light. The teeth were left undisturbed for 6 hrs after fluoride application.
- *Group II–APF gel (FG):* Teeth were exposed to 1.23% APF gel (Patterson NE. International, USA) for one minute with a micro brush. The teeth were left undisturbed for 30 min after fluoride application. APF gel was applied once a week, i.e. two times during the entire period of the experiment.
- *Group III–Fluoride toothpaste (FTP):* Teeth were dried, and the exposed enamel in the box was painted with a pea-size amount of Clinpro™ Tooth Crème Paste (0.21% w/w sodium fluoride anti-cavity paste with Tri-Calcium Phosphate) with a cotton swab. It was left undisturbed for 3 minutes. The Clinpro™ Tooth Crème on each tooth was removed, and toothpaste slurry was made by proportioning the removed paste with three parts distilled water. The teeth were brushed by Power toothbrush (Oral-B® Crossaction® Power, P&G, USA) for 5 sec using the 1:3 toothpaste slurry. The teeth were then left undisturbed for 30 min. This procedure was done twice daily for the period of the experiment.
- *Group IV–Fluoride mouth wash (FMW):* Teeth were immersed in Sodium fluoride 0.044% (w/v)

mouthwash (Colgate® Phos-Flur® Ortho Defense Rinse, Colgate-Palmolive, NY) and rinsed daily for one minute. Teeth were left undisturbed for 30 min.

- *Group V–Control (C):* Nothing was applied.

After finishing all the above-mentioned stages, the samples were rinsed with distilled water for five seconds.

Demineralization Procedure

The daily procedure of pH cycling included a demineralization period of 6 hours and a remineralization period of 17 hours. Each tooth was immersed in 60 mL demineralization solution containing 2.0 mmol/L calcium, 2.0 mmo/L phosphates, and 75 mmol/L acetate at pH 4.3 for 6 at room temperature. Specimens were then removed from the demineralization solution, rinsed with distilled water. Each group specimens were subjected to their respective fluoride regimen and immersed individually in 40 mL of remineralization solution at room temperature overnight (17 hours) to simulate the remineralizing phase of the caries process. The remineralization solution consisted of 1.5 mmol/L calcium, 0.9 mmol/L phosphates, 150 mmol/L potassium chloride, and 20 mmol/L cacodylate buffers at pH 7.0. This cycling procedure was repeated daily for 14 days. All teeth were dried at day 14, and the presence of demineralization was confirmed by the appearance of frosty white enamel and readings were recorded with Diagnodent. The recordings were done at baseline and after completion of the experiment. The data thus obtained were recorded and tabulated and statistically analyzed using the Statistical Package for Social Sciences (SPSS) IBM Inc. 17.0 ver.

STATISTICAL ANALYSIS

Statistical analysis was done for intragroup, intergroup between two groups, and multiple group comparison of DIAGNOdent score. Mean, and the standard deviation was estimated from the samples for each study group, at baseline and after completion of the experiment. The change in values was calculated along with its mean for each group. Normality of data was tested using Shapiro–Wilk Test and non-parametric tests like Kruskal–Wallis ANOVA for intergroup comparison along with Mann–Whitney U test for multiple comparisons and Wilcoxon signed-rank test for intragroup change was used.

RESULTS

Statistical test by Kruskal–Wallis analysis of variance (ANOVA) showed that there was a significant difference in the mean values between the five groups at baseline and after 14 days (Table 1). Mean value at baseline was highest for Group 3 (FTP) 4.050 ± 1.468 followed by group 5 (C) 2.350 ± 1.496, group 2 (FG) 2.450 ± 1.986, group 4 (FMW) 2.400 ± 1.667 and then group 1 (FV) 2.200 ± 0.834. Mean value after 14 days of demineralization and remineralization cycle was highest for group 5 (C) 55.800 ± 36.966 followed by group 4 (FMW) 52.250 ± 34.772, group 2 (FG) 35.250 ± 30.914, group 3 (FTP) 29.250 ± 22.539 and then group 1 (FV) 18.4 ± 10.723 (Table 2).

When statistical comparison of multiple group performance was done taking the control group as standard, the comparison of 14-day mean DIAGNOdent score value change of group 1 (FV) *vs.* group 5 (C) showed high statistical significance ($p < 0.001$) where FV group had considerable low mean DIAGNOdent score value. Similarly, group 2 (FG) vs. group 5 (C) showed a statistically significant lower mean in group 2 (FG). The FTP group also out-performed the control group in comparison, group 3 (FTP) vs. group 5 (C). Though the group 4 (FMW) had slightly lower mean when compared to the control group, it was statistically nonsignificant. group 4 (FMW) *vs.* group 5 (C) (Table 3).

Table 1: Comparisons of mean diagnodent score values between different groups

	Day 1		*Day 14*		*Change*	
Group	*Mean*	*± SD*	*Mean*	*± SD*	*Mean*	*± SD*
1. FV	2.200	0.834	18.400	10.723	16.200	10.773
2. FG	2.450	1.986	35.250	30.914	32.800	30.495
3. FTP	4.050	1.468	29.250	22.539	25.200	22.315
4. FMW	2.400	1.667	52.250	34.772	49.850	35.106
5. Control	2.350	1.496	55.800	36.966	53.450	37.216
P value #	0.002*		0.001*		0.001*	

Kruskal–Wallis ANOVA: *p <0.01; Significant

Table 2: Mean, standard deviation and test of significance of mean diagnodent score values between each group

	Day 1		*Day 14*		*Change*			
Group	*Mean*	*± SD*	*Mean*	*± SD*	*Mean*	*± SD*	*Z value#*	*p value#*
1. FV	2.200	0.834	18.400	10.723	16.200	10.773	3.923	<0.001**
2. FG	2.450	1.986	35.250	30.914	32.800	30.495	3.921	<0.001**
3. FTP	4.050	1.468	29.250	22.539	25.200	22.315	3.924	<0.001**
4. FMW	2.400	1.667	52.250	34.772	49.850	35.106	3.921	<0.001**
5. Control	2.350	1.496	55.800	36.966	53.450	37.216	3.922	<0.001**

Wilcoxon Signed rank test; **p<0.001; highly significant

DISCUSSION

Orthodontic appliances modify the oral ecosystem leading to an increase in the number of cariogenic bacteria. After the placement of fixed appliances into the oral cavity, rapid shift in the bacterial flora of plaque occurs, upsetting the balance between the processes of demineralization and remineralization, thereby increasing the patient's risk of caries and white spot lesions.[7]

In general, the progression and occurrence of white spot lesions are significantly higher in orthodontic patients than non-orthodontic patients, and these WSLs may present esthetic problems years after treatment.[8] It was found in a study that the prevalence of at least one WSL in patients who underwent orthodontic treatment with fixed appliances was 49.6% as compared to only 24% in an untreated control group.[7]

Many preventive agents have been used in literature to prevent WSL's. The favorable role of fluorides as caries preventive agent has been documented for many years. The fluoride ions prevent dental caries by incorporating into the tooth structure and modifying the bacterial metabolism through inhibition of some enzymatic processes, therefore,have an inhibiting effect on tooth demineralization and an enhancing effect on remineralization.[9,10]

There are several methods of fluoride administration to tooth enamel during orthodontic treatment which include fluoridated toothpaste, fluoride-containing mouth rinses, fluoride varnishes, and fluoride-releasing glass ionomer bonding materials. To the best of our knowledge, no study has been done to test the efficacy of various low concentration and high concentration topical fluoride treatment regimens, in the prevention of enamel demineralization. This *in vitro* study was designed to permit an even testing field for comparing various concentrations of topical fluorides.

The results of our study indicated that different fluoride regimens with varied fluoride concentrations had a role to play in producing varied values after laboratory pH cycling challenge. This was in accordance with Benson and Parkin,[11] Chadwick et al.[12] and Alexander, Ripa.[13]All the topical fluoride groups performed significantly better than the control group. This showed that the control group which did not receive any fluoride treatment was left vulnerable to the laboratory pH cycling challenge. This was in accordance with Ogaard et al.[14,15] and O'Reilly et al.[16] who stated that topical fluorides effectively prevent the development and progress of white spot lesions.

When compared to the control group, the 1.23% fluoride gel which was applied only once a week performed significantly better in preventing the enamel demineralization. This was in accordance with Alexander, Ripa.[13] Group 4 (FMW) performed marginally better than the control group but was statistically non-significant This is contrary to the published studies done by Benson and Parkin,[11] Boyd[17] and Shetty et al.[18] who attribute better performance to the NaF mouth wash. This is probably because this is the first time that Wei Hu and Featherstone[19,20] laboratory pH cycling model for testing NaF mouthwash has been used.

Clinpro TM XT VarnisCh (group 1) which is light cured resin-modified glass ionomer that releases calcium, fluoride, and phosphorus, outperformed all the other topical fluoride groups. It showed maximum resistance to demineralization. Our results were in agreement with Jena et al. (2015).[21] and Shruthi et al.[22] Clinpro showed the best remineralization when compared to other varnishes.[23] The remineralization potential of it has been attributed to its manufacturing process that includes milling technique fusing beta-tricalcium phosphate and sodium lauryl sulfate creating a "functionalized" calcium and free phosphate. There are numerous studies in literature proving its effectiveness compared to other fluoride varnishes in preventing caries. Further studies may be required to arrive at a clinically correlatable conclusion.

Limitations

In the present study, demineralization was obtained with the use of chemical products and did not occur due to the presence of Streptococcus mutans and its acid by-products.

Table 3: Between-groups statistical multiple comparisons of diagnodent score using Mann–Whitney U test

	Day 1		*Day 14*		*Change*	
Group	*Z value#*	*p value#*	*Z value#*	*p value#*	*Z value#*	*p value#*
Group 1 *vs* 2	0.426	0.670	1.625	0.104	1.638	0.101
Group 1 *vs* 3	4.008	<0.001**	1.625	0.104	1.232	0.218
Group 1 *vs* 4	0.215	0.830	3.377	0.001*	3.276	0.001*
Group 1 *vs* 5	0.471	0.637	3.367	0.001*	3.344	0.001*
Group 2 *vs* 3	2.713	0.007*	0.095	0.924	0.528	0.597
Group 2 *vs* 4	0.125	0.900	1.905	0.057	1.638	0.101
Group 2 *vs* 5	0.195	0.845	2.137	0.033*	2.017	0.044*
Group 3 *vs* 4	3.017	0.003*	2.159	0.031*	2.248	0.025*
Group 3 *vs* 5	3.306	0.001*	2.324	0.020*	2.559	0.011*
Group 4 *vs* 5	0.014	0.989	0.289	0.772	0.244	0.807

Mann–Whitney U Test; *p <0.05; Significant; **p <0.001; highly significant

This is an *in vitro* study; the results obtained hence need further investigations before deriving clinical correlations. The presence of saliva and its buffering action in the oral cavity may produce varied results for the materials tested.

Only one representative of each of the topical fluoride groups was tested.

CONCLUSION

Even under the limitations of the study, the following conclusions and recommendations are derived from this in vitro study:

- The well-adapted pH cycling model (Wei Hu and Featherstone) successfully produced discernible enamel demineralization in all the tested teeth, which was quantified and evaluated with the help of DIAGNOdent.
- All the topical fluorides tested were able to reduce the enamel demineralization adjacent to orthodontic brackets when compared to the control group under similar testing conditions, but to a varying degree.
- The least change in the mean DIAGNOdent score values from baseline to 14 days was seen in group 1 fluoride varnish (FV), followed by group 3 fluoride toothpaste (FTP), group 2 fluoride gel (FG), group 4 fluoride mouthwash (FMW) and then the group 5 control (C). The lowest mean value indicates the maximum resistance to enamel demineralization.

CLINICAL SIGNIFICANCE

Our investigation showed that the prevention of demineralization was seen after application of all the products but light cured fluoride varnish was best among all the groups. The fluoride toothpaste was also an effective, low cost and readily available option to prevent enamel demineralization around orthodontic brackets. Therefore, orthodontists should advice all the patients to use fluoridated products and should monitor them regularly for the development and progression of any white spot lesion in them.

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