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The efficacy of a fluoride varnish in reducing enamel demineralization adjacent to orthodontic brackets: an *in vitro* study

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Structured Abstract

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Objectives – To test the hypothesis that fluoride varnish is effective in reducing demineralization (white spot) lesions adjacent to bonded orthodontic brackets.

Design – Two similar samples of extracted bovine incisors, with bonded orthodontic brackets, were separated into an experimental group (fluoride varnish was applied) and control group (no fluoride varnish) to examine the preventive effects of fluoride varnish.

Setting and Sample Population – The dental clinic of the State University of Maringá – UEM (Maringá, Paraná, Brazil). Thirty-eight extracted bovine incisors with bonded orthodontic brackets.

Experimental Variable – Fluoride varnish was applied topically to half of the sample of extracted bovine teeth. No varnish was applied to the other half.

Outcome Measure – The depths of enamel demineralization (white spot) lesions were measured from polarized light microscopy images using image analysis software.

Results – The teeth in both the experimental and control groups had been exposed to a cariogenic environment twice a day for 35 days. Those teeth that had been treated with two applications of fluoride varnish (one at the outset and another 15 days later) demonstrated about 38% less mean lesion depth than teeth where no varnish had been applied.

Conclusion – Orthodontists may wish to consider the application of fluoride varnish during fixed orthodontic therapy to help reduce the development of enamel white spot lesions.

Key words: demineralization; fluoride varnish; orthodontic brackets; white spot lesions

Introduction

Orthodontic treatment with fixed appliances predisposes patients to larger accumulations of bacterial plaque and, therefore, to demineralized enamel 'white spot' lesions. Considering the mechanical difficulties of removing plaque with orthodontic brackets in place, compliance with proper oral hygiene is critical. Unfortunately, patient compliance is a commodity that is unpredictable and decreasing. Consequently, the incidence of enamel decalcification and caries during orthodontic care is increasing. For example, Gorelick *et al.* (1) found white spot lesions for nearly 50% of patients that underwent orthodontic treatment. In addition, Ogaard *et al.* (2) reported that these lesions can develop as quickly as only 4 weeks or the average time between orthodontic visits. For a specialty whose objectives are to improve facial and dental esthetics, the presence of unsightly white spot lesions may detract from the beneficial effects of orthodontic treatment.

Orthodontists have long-attempted to reduce demineralization with limited success. For instance, the beneficial effects of dentifrices and/or home use of fluoride solutions have been confirmed (3); however, patient adherence to prescribed use of these materials is also problematic. Geiger *et al.* (4, 5) observed that 52.5% of the patients did not comply with the home use of fluoride solutions.

Preventive measures that do not require patient compliance would seem to make more sense for the typical orthodontic patient population: adolescents who already have a higher incidence of dental caries. Todd et al. (6) reported that the application of fluoride varnish (Duraflor®; Pharmascience Inc., Montreal, Canada) promoted 50% less enamel demineralization. This fact was corroborated by Ogaard *et al.* (7) (reduction of 48%), and recently, Vivaldi-Rodrigues *et al.* (8) observed a 44% reduction in the incidence of white spot lesions with tri-monthly application of fluoride varnish after 12 months of corrective orthodontic treatment.

Teeth that had fluoride varnish applied around composite resin-bonded brackets showed a 35% reduction in demineralized lesion depth (9). In contrast, teeth with RMGI-bonded (resin-modified glass–ionomer cement) brackets demonstrated 50% reduction in lesion depth whether or not fluoride varnish was applied. RMGI adhesives have been demonstrated to sustain fluoride release long after initial application (10) but they only protect a limited area immediately adjacent to the orthodontic bracket (11, 12). In addition, bond failures with RMGI have been found to be similar (13) or worse than composite resins (4, 14).

The application of fluoride varnish is a preventive protocol that does not require patient compliance and permits the orthodontist to benefit from the bond strength of composite resins. Prolonged contact time with fluoride varnish permits significantly more incorporation of fluoride than other cooperation-based fluoride applications (e.g. acid phosphate fluoride gel, monofluoridephosphate dentifrices, home fluoride rinses) (15–19). For instance, Peterson *et al.* (20) observed that a tri-monthly application of fluoride varnish resulted in a dramatic reduction in caries incidence (21) and the application of a fluoride varnish can be easily adapted to current orthodontic bonding techniques (22–24).

The purpose of this *in vitro* study was to evaluate the efficacy of fluoride varnish in preventing enamel demineralization lesions adjacent to orthodontic brackets bonded with composite resin.

Materials and methods

Forty incisor teeth were collected from the mandibles of a 3-year-old Nelore cattle and immediately stored in a 0.1% thymol solution. Each tooth was subsequently disinfected with a 0.12% chlorexidine solution and the residual calculus, bone, and soft tissue were removed with a no. 15 blade. The enamel surface of all teeth was then cleansed with a mixture of pumice and distilled water using a Robinson prophylaxis brush on a slow speed hand piece. All teeth were finally rinsed with distilled water and designated at random into two equal groups of 20 teeth.

A window, the size of an orthodontic bracket base, was cut out from a piece of adhesive tape. The tape was placed with the window centered on the facial surface on a tooth to limit acid etching of the entire enamel surface; unintentionally initiating enamel demineralization. The enamel, exposed by the window in the tape, was etched for 30 s with 35% phosphoric acid gel, rinsed with distilled water for the same amount of time, and thoroughly dried with compressed air.

Metal orthodontic brackets (Morelli, Sao Paulo, Brazil) were bonded to all teeth using a chemically cured composite bonding resin (Concise; 3M Unitek, Monrovia, CA, USA) following the manufacturer's instructions. Excess resin flash was removed from around each bracket base with a dental scaler. Twenty minutes after bonding, the adhesive tape was removed from each tooth and any excessive adhesive residue from the tape was removed with methanol.

A box was drawn with a HB graphite pencil around each bracket with the perimeter of this box 2 mm from the bracket margin. All teeth were then painted with a thin coat of acid-resistant (non-fluoridated) varnish on all surfaces except the 2 mm area between the bracket and the box that was drawn around the bracket.

The 40 teeth with their bonded brackets were randomly distributed into two equal groups. The teeth in group 1 ($n = 20$) received no further treatment while those in group 2 ($n = 20$) were dried and the exposed enamel within the lines of the box (adjacent to the bracket base) was painted with a thin layer of Duraflor fluoride varnish (Pharmascience Inc.).

After allowing the varnish to dry for 5 min, all teeth in both groups 1 and 2 were stored in separate beakers of a 200 ml artificial saliva solution consisting of 20 mmol/l $NaHCO_3$, 3 mmol/l NaH_2PO_4, and 1 mmol/l $CaCl_2$, at neutral pH. After 12 h, cycling between artificial saliva and an artificial caries solution began. Twice daily, with a 6-h interval, all teeth were immersed for 1 h in beakers (one for each of the two experimental groups) containing 200 ml of artificial caries solution (2.2 mmol/l Ca^{+2}, 2.2 mmol/l PO_4^-, 50 mmol/l acetic acid at pH 4.4). Both solutions were stored in an incubator at a constant temperature of 37°C and were changed every 3 days during the experimental period of 35 days.

After 1 h of exposure to the caries challenge, all teeth in each group were removed, rinsed with de-ionized water, and brushed for 5 s on each surface using a Colgate Classic soft-bristled toothbrush, without dentifrice, to simulate mechanical wear of the varnish material. As a result, each tooth in both groups was 'brushed' twice daily. The teeth were cycled between the artificial saliva and caries challenge following this protocol for 35 days. Fluoride varnish was reapplied to only the teeth in group 2 on day 15.

On day 35, all teeth were removed from the saliva solution, dried thoroughly, and the brackets were removed. Buccolingual longitudinal sections of approximately 400 μm were made of each tooth using a water-cooled diamond disk (0.2 mm section; KG Sorensen, Sao Paulo, Brazil). The sections were then reduced to a thickness of 200 μm using wet sandpaper (400, 3M, 211Q, Wetordry, Sumare, SP, Brazil). They were then rinsed with de-ionized water and stored in separate containers (with labels identifying the teeth from each group) of de-ionized water.

When ready for examination, each tooth section was dried with absorbent paper and placed on a histological slide for evaluation under polarized light microscopy using an Olympus BX50 microscope with a 3CC Pro Series digital camera attached to it. Photomicrographs were made at 20× magnification with maximum illumination. Image Pro-Plus (V. 1 Media Cybernetics, Silver Spring, MD, USA) software was used to analyze the digital photomicrographs. This software projects a reference line on the computer screen representing 500 μm. This line was traced onto tracing acetate to create a template for measurement of each enlarged digital photomicrograph. Three vertical lines were drawn on the acetate at 250 μm intervals, perpendicular to the horizontal reference line; thereby, separating the area below the 500 μm line into three equal sections for measurements (L1, L2, L3; Fig. 1).

The tracing acetate template was centered over the enamel lesion on the computer screen with the horizontal reference line superimposed on the image of the enamel surface and the center vertical line (L2) bisecting the length of the lesion. Within the area below the reference line, three depth measurements (in μm) were taken at each of the perpendicular lines (L1, L2, L3) for each lesion.

After 1 week, the same investigator repeated the three measurements for each tooth. The average of the two measurements made at each of the three perpendicular lines was then calculated. Thxe three depth measurements for each lesion were then averaged to give the mean demineralization depth for that tooth. The mean maximum depth measurement for each

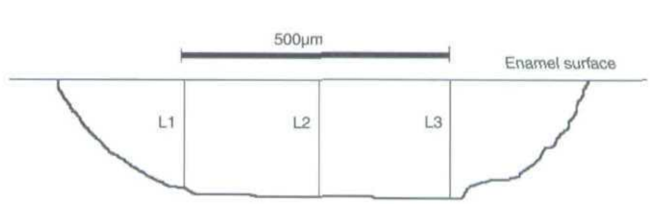

Fig. 1. Template used to measure the lesion depths.

group was calculated from the measurements of maximum lesion depth made for each tooth. Differences in mean lesion depth and mean maximum lesion depth between group 1 and group 2 were then analyzed.

Results

Each of the two experimental groups had one tooth that was lost during the sectioning process, so both groups ended with 19 teeth (n = 38). The mean decalcification depths for group 1 (control) and group 2 (varnish) are shown in Table 1. It is important to note that every tooth, control and experimental, demonstrated some level of enamel demineralization.

Analyzing the data with the Student's *t*-test, there were statistically significant differences between the two groups ($p < 0.01$). Specifically, those teeth that did not receive fluoride varnish (group 1) demonstrated, on average, 38% deeper demineralization lesions (Fig. 2) and the mean *maximum* lesion depth was also deeper in the control group (209.958 μm) when compared with teeth that had been treated with fluoride varnish (150.291 μm) (Table 2, Fig. 3). Figures 4 and 5 represent the typical lesions found in the control and the test groups, respectively.

Table 1. **Comparison of mean decalcification depths between the control and experimental groups using the Student's *t*-test**

Group	Group 1 (control)	Group 2 (varnish)	*p*
Mean	173.2384	108.3108	0.003*
SD	77.90322	64.79095	

*Statistically significant ($p < 0.01$).

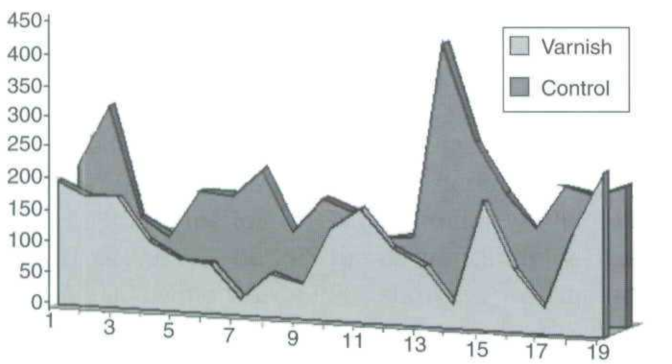

Fig. 2. Graph comparing the mean lesion depth of the control and experimental groups. The average depth of demineralization was greater for teeth that did not receive treatment with fluoride varnish.

Table 2. **Comparison of the mean values of the *maximum* decalcification depths between the control and experimental groups using the Student's *t*-test**

Group	Group 1 (control)	Group 2 (varnish)	*p*
Mean	209.9584	150.2912	0.005*
SD	92.0585	73.8283	

*Statistically significant ($p < 0.01$).

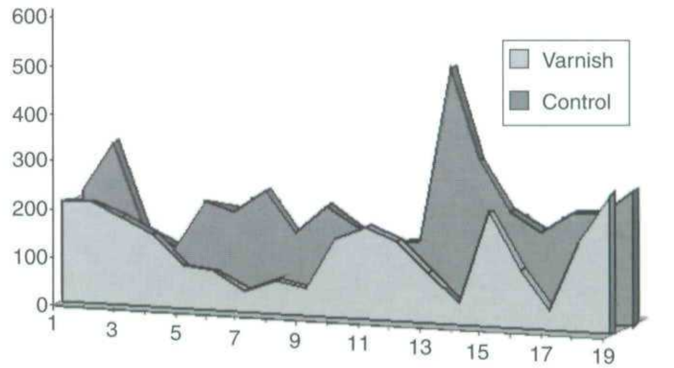

Fig. 3. Graph comparing the maximum lesion depth of the control and experimental groups. The average maximum lesions depths were greater for teeth that did not receive treatment with fluoride varnish.

Discussion

During the course of orthodontic treatment, bonded brackets promote more retention of dental plaque and make oral hygiene difficult. This combination creates a propitious environment for the development of white spot lesions (4–7), especially when patients fail to comply with oral hygiene and/or the use of fluoride rinses or gels. The prevalence of white spot lesions in patients who seek orthodontic treatment is in the range of 50–96% (4, 5, 7, 8).

The development of these lesions can occur rapidly (around 4 weeks) and when these lesions reach advanced stages they will no longer spontaneously decalcify (7). Therefore, it would be extremely valuable if early diagnosis of these lesions were possible. Unfortunately, by the time that lesions are clinically visible, the damage is often already irreversible and unsightly.

One preventive method has been the use of home fluoride rinses and professionally applied fluoride gels.

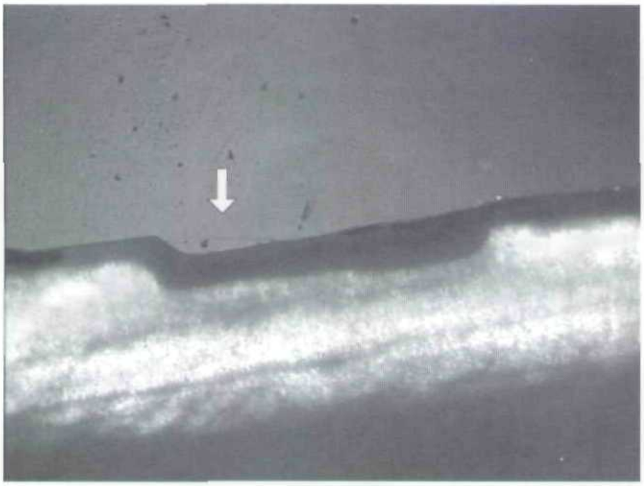

Fig. 4. Polarized light photomicrograph of an enamel lesion adjacent to the site of an orthodontic bracket on a tooth that did not receive fluoride varnish (control group) demonstrating significant mineral loss (arrow).

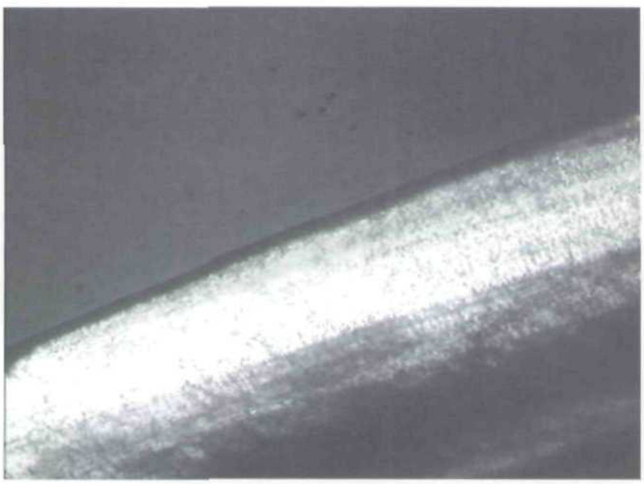

Fig. 5. Polarized light photomicrograph of enamel adjacent to the site of an orthodontic bracket on a tooth that received fluoride varnish treatment (experimental group). On average, about 38% less enamel demineralization was noted when the fluoride varnish was used.

The home use of 0.05% sodium fluoride solution has been proved to significantly reduce the incidence of white spot lesions but will not completely inhibit their appearance (3–5, 7, 25). Unfortunately, the patient that would benefit most from a fluoride rinse (i.e. one who is non-compliant with oral hygiene) is also the one least likely to adhere to the prescribed use of these rinses (4, 5).

Fluoride varnishes, 5% sodium fluoride in a colophony base, produce 40–50% reduction in white spot appearance and require no patient compliance as they are placed by the orthodontist (6–9, 11, 25). In spite of this positive result, varnishes also cannot completely prevent these lesions (6–9). In the present study, this fact was confirmed as lesions still developed on not only the control teeth but also on those that had been treated with fluoride varnish (Fig. 2). More importantly, the fluoride varnish did reduce the average depths of lesions by about 38% (Table 1) when compared with teeth that did not receive the varnish.

Although there are some differences between the bovine teeth used in this study and human enamel (26, 27) (e.g. decalcification occurs more quickly in bovine enamel), Featherstone and Mellberg (25) reported that bovine teeth were suitable for comparative purposes in demineralization studies such as the present one. To account for the differences in bovine enamel, the present study was conducted over a short period of time (35 days) and fluoride varnish was applied twice during the experimental period.

Cycling between periods of demineralization (caries solution) and remineralization (artificial saliva solution) during this experiment was intended to simulate a clinical situation. Within the normal oral environment, there are periods of higher caries challenge; dependent upon the eating habits of each person. Demineralization occurs when the pH of the mouth becomes more acidic but there is also subsequent remineralization during the longer periods of exposure to saliva during the rest of the day. In the present study, an artificial saliva solution was used to provide the calcium and phosphate necessary to promote this same type of naturally occurring remineralization.

This *in vitro* study showed that the application of the fluoride varnish around orthodontic brackets bonded to the buccal surface of bovine incisors resulted in an approximate 38% reduction in the depth of enamel demineralization (Tables 1 and 2, Figs 4 and 5); thereby, confirming results reported by Schmit *et al.* (9) This reduction in lesion depth is due to two factors: 1) the initial protective coating of the varnish, and 2) reduced enamel solubility due to the uptake of fluoride from the varnish. The physical barrier protection from a fluoride varnish is temporary as this material is easily abraded way during typical tooth brushing. The protection from the fluoride, incorporated into the surface of the enamel from the varnish, also diminishes with time and as a result, reapplication of this material is recommended at least every 3 months (7).

Conclusion

Analyzing the measurements obtained from photomicrographs of enamel sections of bovine teeth, it may be concluded that fluoride varnish (Duraflor®) promotes a reduction of about 38% in the mean depth of enamel demineralization lesions adjacent to orthodontic brackets bonded with composite resin.

Considering the results of this and many other studies that have demonstrated the efficacy of fluoride varnish in reducing the incidence and depth of enamel demineralization, orthodontists should consider its routine use in clinical practice, especially for patients exhibiting poor oral hygiene.

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