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Varnish or Polymeric Coating for the Prevention of Demineralization? An *Ex Vivo* Study

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Abstract

Objective: The ability of an experimental coating, Odyssey, to prevent demineralisation ex vivo was compared with that of a fluoride varnish, Duraphat® and a chlorhexidine-containing varnish, Cervitec.

Design: an ex vivo single-blind study.

Setting: Hard tissue research laboratory.

Materials and methods: thirty bovine enamel blocks 0·5 cm × 1·5 cm were divided into 6 groups of 5 specimens. The enamel blocks were then allocated to one of 6 surface treatments.

Interventions: (1) surface left unprepared (control), (2) Duraphat® application, (3) Cervitec application, (4) experimental polymer coating, (5) enamel conditioned with 10% citric acid and coated with the experimental polymer coating Odyssey (O + C), (6) enamel etched for 30 sec with 37% phosphoric acid and coated with the experimental coating (O + E). All specimens were cycled for 7 days through a daily procedure of demineralisation for 4 hours and remineralisation for 20 hours, and exposed to an equivalent of 2 months toothbrushing. A single operator blinded to the treatment allocation of each specimen carried artificial lesion depth assessment out using computer-assisted transverse microradiography.

Results: The control group had the greatest mean lesion depth (97·16 + 29·8 μm) with the Duraphat® group exhibiting the lowest mean lesion depth (24·53 + 15·44 μm). The Duraphat®, Odyssey, O + C and O + E groups all had significantly less lesion depth when compared with no surface preparation ($p < 0·05$ for all comparisons). There were no significant differences between any of the Odyssey groups.

Conclusions: The efficacy of Duraphat® application in preventing demineralisation ex vivo has been demonstrated in the present study, but clinical trials are required to assess its usefulness in orthodontic practice.

Index words: Demineralisation, Ex vivo, Polymeric Coating, Varnish.

Introduction

It is well known that fixed orthodontic appliances predispose to increased plaque accumulation and enamel demineralization may occur within a few weeks of appliance placement (Gwinnet and Ceen, 1979; Gorelick *et al*., 1982; O'Reilly and Featherstone, 1987). One solution to this problem is to provide a low dose fluoride application, either via self-administration or professional means (Geiger *et al.,* 1988). Self-administration methods, such as fluoride mouth rinsing, are effective at reducing demineralization, but patient compliance is poor (Geiger *et al*., 1992). Professionally applied methods have included the incorporation of fluoride into the composite used for bracket bonding, although the clinical efficacy of these bonding agents in the reduction of demineralization remains equivocal (Sonis and Snell, 1989; Underwood *et al*., 1989; Øgaard *et al*., 1992; Mitchell, 1992; Turner, 1993; Trimpeneers and Dermaut, 1996).

It has been suggested that conventional glass ionomer cements when used for orthodontic bonding may reduce demineralization (Marcusson *et al*., 1997), but their weak bond strengths make them unreliable for routine use (Millett and McCabe, 1996). However, the more recently introduced hybrid glass ionomer cements have improved clinical performance (Silverman *et al*., 1995; Chung *et al*., 1998), although their fluoride releasing potential is dependent on both material and local factors (Valk and Davidson, 1987; Twetman *et al*., 1997; Monteith *et al*., 1999).

Other alternatives have been the application of a polymer or coating to the labial enamel surface (Frazier *et al*., 1996). A clinical trial has also shown that the application of

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light-cured resin sealants to the labial enamel surface has been found to reduce the extent of demineralization by 13% (Banks and Richmond, 1994). In that study, no objective account would appear to have been taken of the effect of any chemical attack or abrasion likely to occur within the oral environment. Finally, the use of fluoride varnish application has been investigated and this has been shown to permit a prolonged fluoride exposure compared with a mouthrinse resulting in an increased enamel fluoride uptake (Petersson, 1993). For example, an *in vitro* application of a fluoride varnish Duraphat® had similar cariostatic ability to bonding brackets with a conventional glass ionomer cement, leading to a significant reduction in demineralization compared with the controls (Kindelan, 1996; Todd *et al.*, 1999).

The aim of this study was to compare the ability of an experimental coating to prevent demineralization *ex vivo* with that of a fluoride varnish, Duraphat® and a chlorhexidine-containing varnish, Cervitec.

Materials and methods

Thirty incisor teeth were collected from sub 2-year-old bovine mandibles; all teeth were stored in a saturated thymol solution for at least 6 months before further preparation. Each tooth was decoronated, the pulp extirpated, and the pulp canal sealed with sticky wax. The labial surface was then cleaned with a mixture of pumice and water, and dried in a stream of air. An enamel block 0·5 × 1·5 cm was cut in an inciso-cervical direction using a Labcut 1010 (Agar Scientific Limited, Stansted, Essex, England) from the labial aspect of each incisor (Figure 1). Each block was coated with a layer of acid-resistant nail varnish (Proctor and Gamble, Weybridge, Surrey, UK) on all sides, but leaving half of the labial surface of the enamel block exposed, the varnished half acting as control (Figure 2).

The enamel blocks were then allocated to one of six possible treatments to the exposed labial enamel. There were five enamel blocks in each group. This sample size has been used previously and demonstrated adequate power to detect a difference in demineralization between fluoride and non-fluoride treatments (Øgaard *et al.*, 1988a,b):

- Group 1—enamel surface untreated (control);
- Group 2—a single application of Duraphat® varnish (Colgate-Palmolive (UK) Ltd., Guildford, Surrey, England);
- Group 3—a single application of Cervitec (Vivadent, Liechtenstein);
- Group 4—a single application of an experimental polymer coating, 'Odyssey' (3M, Unitek, Monrovia, CA, USA), to the untreated enamel;
- Group 5—the enamel pre-conditioned with 10% citric acid for 30 seconds, followed by a single application of Odyssey (O+C);
- Group 6—the enamel etched for 30 seconds with 37% phosphoric acid and coated with a single application of Odyssey (O + E). In Groups 5 and 6, the acids were removed by washing specimens in distilled water for 1 min, followed by drying in a stream of air for 1 minute.

Prepared specimens were allowed to set in a humid atmosphere for 12 hours at 37°C. The exposed labial enamel surface was then brushed for 8 minutes with a non-fluoridated toothpaste slurry using a Braun Oral-B Plaque Remover 3D electric toothbrush, applied according to manufacturer's instructions. Each specimen was secured in a clamp and the electric toothbrush was suspended from a tripod with a 25 g weight attached to the brush head. This standardized the brushing force for all specimens. An 8-minute brushing duration has been estimated to simulate the equivalent of 2 months brushing for a single tooth surface (Donly *et al.*, 1997).

Each specimen was then rinsed with de-ionized water and dried thoroughly. The blocks were subsequently exposed to a daily cycle of de- and remineralization (Creanor *et al.*, 1998). The demineralizing solution contained 2·0 mM calcium, 2·0 mM phosphate, and 50 mM glacial acetic acid and was adjusted to pH 4·6 by the addition of 1·0 M sodium hydroxide. The remineralizing solution contained 2·0 mM calcium chloride and 2·0 mM sodium dihydrogen orthophosphate and was adjusted to pH 6·8 by the addition of 0·1 M sodium hydroxide. Each specimen was immersed for 4 hours per day in 10 ml of demineralization solution, again at 37°C, before being rinsed and returned to fresh remineralizing solution for the remaining 20 hours. This cycle continued for 7 days, at the end of which the nail varnish was removed from all surfaces of the specimens with acetone. The mid-region of each specimen was subsequently sectioned perpendicular to its

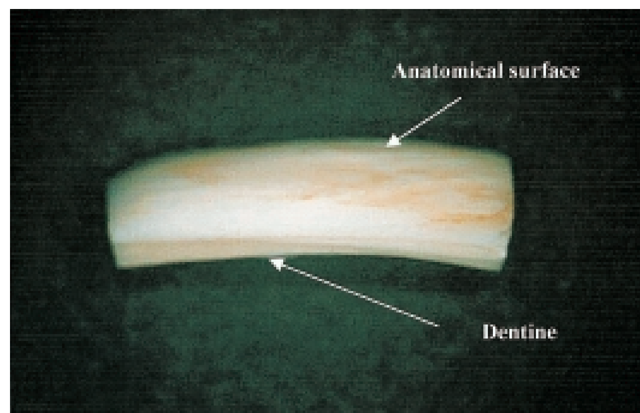

Fig. 1 Unprepared bovine slab showing enamel and dentine.

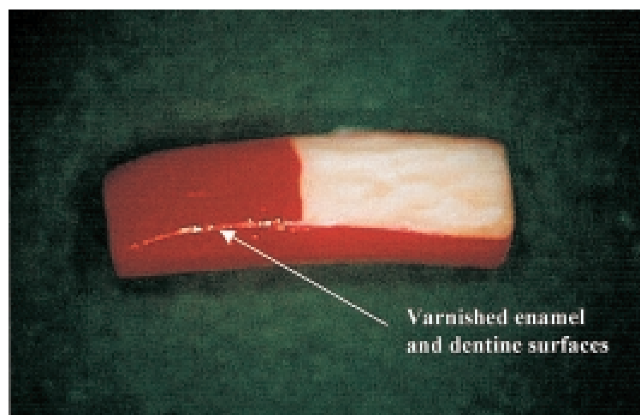

Fig. 2 Varnished bovine slab indicating that all surfaces covered apart from half of the natural anatomical enamel surface of the specimen.

long axis using a Microslice 2 precision slicing machine (Malvern Instruments, Malvern, UK), and was then hand lapped to a final measured thickness of 130–145 μm. Two sections were cut per specimen.

In preparation for transverse microradiography (TMR), sections were mounted between two sheets of Clingfilm and placed along with an aluminium step wedge for calibration on high resolution radiographic film (Kodak film, SO343, Eastman Kodak, Rochester, NY, USA). The film and sections were mounted in light-tight holders and were exposed to Cu-K∞ X-rays for 10 min at 20 kV and 30 mA. Following film processing, microdensitometry was carried out using a PC-based image analysis system (Brian Reece Scientific, Berkshire, UK) by a single operator who was unaware of the treatment allocation of each specimen. The film was examined under a light microscope (Leitz Ortholux II, Milton Keynes, UK) and the black and white image of any artificial lesion detected was transmitted via a CCD video camera (Cohu, San Diego, CA, USA) to a frame grabber. A typical area of subsurface enamel demineralization is illustrated in Figure 3. The image analysis software (Brian Reece Scientific, Berkshire, UK) then produced a quantitative mineral profile across the area of the section scanned. For all sections, the parameter chosen for comparison was the maximum lesion depth. This is defined as the distance from the anatomical surface through the demineralized area to the point where normal enamel mineral content is reached (Figure 3).

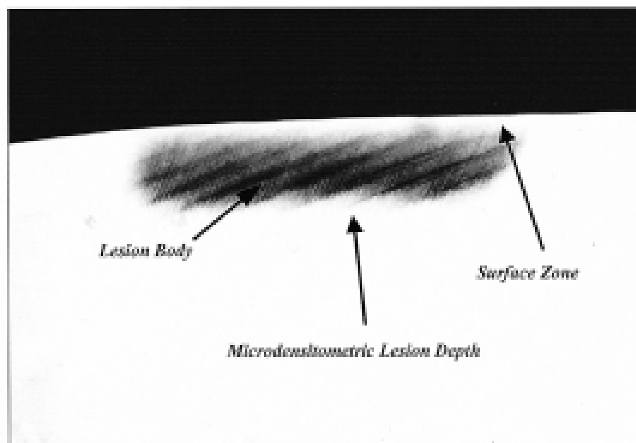

FIG. 3 Microradiograph of a typical subsurface enamel demineralization.

Statistical analysis

Due to the need for comparison between six groups, analysis of variance was used to determine if any significant differences in mean lesion depth existed between specimen groups. A Tukey test was then used to identify those that were significantly different.

Results

The mean (SD) of lesion depth for each enamel preparation group is given in Table 1. The table of the analysis of variance is given in Table 2 and between group comparisons given in Table 3. The control group had the greatest mean lesion depth (97·16 + 29·8 μm) with the Duraphat® group exhibiting the lowest mean lesion depth (24·53 + 15·44 μm). The Duraphat®, Odyssey, O + C and O + E groups all had significantly less lesion depth when compared with no surface preparation ($P < 0{\cdot}05$ for all comparisons). There were no significant differences between any of the Odyssey groups.

Discussion

The application of Duraphat® to enamel slabs, which were then subjected to the equivalent of 2 months tooth brushing, significantly reduced demineralization compared with

TABLE 2 *One-way analysis of variance of the depth data, listing the source, degrees of freedom (DF), the sums of squares (SS), the mean of the sums (MS), the variance ratio (F), and the probability (P)*

Source	DF	SS	MS	*F*	*P*
Protocol	5	37194	7439	17·69	<0·001
Error	54	22702	420		
Total	59	59896			

Analysis of variance for depth.

TABLE 1 *Mean (SD) of lesion depth for each group in μm*

	Control	Duraphat®	Cervitec	Odyssey	O + C	O + E
Mean depth (μm)	97·16	24·53	85·69	54·01	53·16	41·61
SD	29·80	15·44	16·77	23·74	10·06	21·22

(O + C, Odyssey plus citric acid conditioner; O + E, Odyssey plus phosphoric acid etchant).

TABLE 3 *Statistical significance values for comparisons between groups*

	Control	Duraphat®	Cervitec	Odyssey	O + C	O + E
Control		<0·001	0·302	0·002	<0·001	<0·001
Duraphat®			<0·001	0·004	<0·001	<0·001
Cervitec				0·003	<0·001	<0·001
Odyssey					0·920	0·230
O + C						0·140

(O + C, Odyssey plus citric acid conditioner; O + E, Odyssey plus phosphoric acid etchant).

all other enamel preparations, including the application of an experimental coating. This finding was similar to that of a study by Todd *et al*. (1999), who found similar results when Duraphat® varnish was compared with a non-fluoridated varnish.

In the study reported here, the specimens were first coated with Duraphat® varnish and then left for 12 hours in a humid environment prior to tooth brushing. This method was adopted in an effort to simulate the usual instructions issued to patients where Duraphat® varnish has been applied, i.e. to refrain from tooth brushing until the morning following the day of application. Todd *et al*. (1999) have used a similar time period.

The use of tooth brushing to simulate mechanical wear in the oral environment is commonplace in studies assessing wear of restorative materials (Hotta and Hirukawa, 1994; Donly *et al*., 1997). Todd *et al*. (1999) used manual tooth brushing twice daily without toothpaste for 37 days, while in the present study, an Oral B electric toothbrush with a non-fluoridated toothpaste was used for the equivalent of 2 months tooth brushing. This time period was chosen to adhere to manufacturer's recommendations that the experimental coating be re-applied no less frequently than every 3 months, a 2-month time frame lying safely within this interval. The powered toothbrush used in this study was applied to the enamel surface of each specimen according to manufacturer's instructions using a force of application to the brush head of 25 g (Donly *et al*., 1997).

The chlorhexidine varnish group showed no significant difference in demineralization when compared with the control group. As a result, it appears that chlorhexidine varnish acts primarily as a mechanical barrier and seems to be removed easily with tooth brushing, leaving the exposed enamel open to the acidic challenge. Nevertheless, caution must be exercised in making this conclusion because this study assessed only the ability of the chlorhexidine varnish to act as a mechanical barrier to the acid attack and did not assess its anti-microbial efficacy.

When we considered the effect of the experimental coating, this had a greater effect than both the control or chlorhexidine varnish groups, although it was significantly inferior to Duraphat®. There are two possible explanations for this. First, the experimental coating may have greater wear resistance than the other coatings. Thereby, acting as a better barrier than either the control or the chlorhexidine varnish groups. Secondly, the experimental coating contained a lower level of fluoride than Duraphat® varnish, Therefore, its ability to prevent demineralization is less than that of Duraphat®, but better than the control and chlorhexidine varnish groups. Observing that the experimental group, where the enamel surface was etched prior to application of the coating, exhibited significantly less demineralization than the other two experimental groups strengthens the case for the second option. This also supports the reported improved uptake of fluoride on etched enamel.

The use of bovine samples requires further comment. Whilst it has been shown that the rates of demineralization vary between human and bovine tissues (Featherstone and Mellberg, 1981), either material can be used for *in vitro* de- and remineralization studies (Featherstone *et al*., 1986). Importantly, as with any *in vitro* test, care must be taken before extrapolating to the *in vivo* situation (Edmunds *et al*., 1988).

Conclusions

The efficacy of Duraphat® application in preventing demineralization *ex vivo* has been demonstrated in the present study, but clinical trials are required to assess its usefulness in orthodontic practice.

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