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Fluoride-releasing elastomeric ligatures assessed with the *in situ* caries model

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SUMMARY The *in situ* caries model was used to assess the clinical benefit of fluoride-releasing elastomeric ligatures. The design of the experiment was a longitudinal, prospective, randomized, crossover clinical trial. Fourteen individuals starting orthodontic treatment with fixed appliances were recruited. Three *in situ* enamel specimens, with pre-formed subsurface carious lesions, were prepared for each patient, from human premolars. Two were placed in customized holders and one was retained as a control specimen. A crossover design was used so that patients had two experimental periods of six weeks with either fluoridated or non-fluoridated elastomerics, and a washout between. Elastomerics were randomly allocated at the first visit and one enamel specimen was placed at the beginning and collected at the end of each experimental period. The specimens were sectioned and ground to 100 μm, and transverse microradiography was carried out. They were analysed using dedicated computer software. The outcome measure was the change in the parameters of the pre-formed carious lesion, expressed as mineral loss (ΔZ), lesion depth (l_d), lesion width (l_w), and ratio ($\Delta Z/l_d$) between the three specimens.

The mean mineral loss (vol %.μm) for the control specimen was 403.7 (±139.5), compared with 599.3 (±515.4) for the non-fluoridated ligatures and 477.2 (±298.4) for the fluoridated ligatures. A one-factor within subjects ANOVA showed no statistical difference between the groups ($P = 0.376$). Fluoride-releasing ligatures do not provide a significant anti-cariogenic benefit in patients undergoing orthodontic treatment. This may be due to the short-term nature of the fluoride release. However, they might affect the local environment surrounding the bracket.

Introduction

Demineralization of enamel surrounding orthodontic brackets is a significant clinical problem. Iatrogenic white spot lesions lead to poor aesthetics and in severe cases the need for restorative treatment. Fluoride mouth rinses have been shown to be effective at reducing the prevalence of enamel demineralization (Geiger *et al*., 1988), however, home compliance is often judged to be poor.

Elastomeric ligatures containing tin fluoride have recently been developed. Wiltshire (1996) stated that, *in vitro*, the release of fluoride is sufficient, even at six months, to inhibit demineralization and promote remineralization. A recent clinical trial (Banks *et al*., 2000) concluded that there was a significant reduction in white spot lesions following the use of fluoride-releasing elastomerics.

The purpose of this study was to examine the clinical benefit of a fluoride-releasing elastomeric ligature, using the *in situ* caries model. This model has been used for a number of years in the study of enamel demineralization (Manning and Edgar, 1992) and has recently been adapted for use in the orthodontic patient (Benson *et al*., 1999). The technique involves the use of enamel specimens from extracted, human premolar teeth. An area of early artificial enamel caries is created on the buccal surface of the extracted tooth. This is removed and divided into two specimens approximately 4×2 mm in size, which can be placed in the mouth. Before placement in

the mouth, a thin section is removed from each experimental enamel specimen to act as a control. After a period in the mouth the specimens are removed, sectioned, and analysed. Any changes in the parameters measuring the extent of the artificial lesion can be compared with the control.

The advantages of the model have been outlined previously (Zero, 1995). Briefly, the model is used in the mouth and includes all the elements that contribute to the caries process, namely a tooth substrate, dental plaque, a carbohydrate challenge, and time; however, the natural caries process is imitated without causing irreversible damage to the participant. Because the specimen has an early lesion, either further demineralization or remineralization can be studied. The model provides adequate controls and allows flexibility in experimental design to permit crossover studies. The trial can be conducted over a relatively short period, avoiding many of the ethical and cost problems of long-term clinical trials. The *in situ* model has two additional advantages for the investigation of orthodontic demineralization: the model will not affect orthodontic treatment and can be used at any stage of therapy.

The disadvantage of the *in situ* model is that there is a considerable amount of laboratory and analytical work involved. For this reason, the number of subjects is usually limited to between five and 40 (Zero, 1995).

The aim of the study was to carry out a longitudinal, prospective, crossover clinical investigation into the clinical effectiveness of fluoride-releasing elastomeric ligatures. The null hypothesis was that there was no difference in the parameters of integrated mineral loss, minimum mineral content, width, and depth of lesion within an individual, when they were supplied with fluoride-releasing elastomeric ligatures compared with conventional non-fluoridated ligatures.

Materials and methods

Laboratory methods

The pre-formed enamel lesions were prepared using a technique described by Leach *et al.* (1989). Premolar teeth extracted for orthodontic purposes were collected and stored in distilled water containing a few grains of thymol. The teeth were carefully examined and those with signs of caries or damage to the enamel were excluded. Selected teeth were lightly abraded with fine abrasive paper (English Abrasives P320A, London, UK) to remove the outermost enamel and remnants of the pellicle from the buccal surface, then degreased using acetone. The teeth were varnished with three coats of acid-resistant nail varnish (Max Factor, Procter and Gamble, Weybridge, UK) except for a window approximately 9×4 mm on the buccal surface. They were mounted on glass rods using inlay wax and immersed into an acidified gel (0.1 M lactic acid, 0.1 M sodium hydroxide, and 6% w/v hydroxyethylcellulose, pH 4.25) for seven days at room temperature.

After removal from the gel, the teeth were washed in distilled water and the varnish removed with acetone. The teeth were examined for visual evidence of demineralization and discarded if there was none. The teeth were mounted individually onto a jig and a block of enamel containing the lesion was cut from the crown of the tooth, together with a margin of sound enamel, using a diamond wire saw (Well type 33 winding unit, W. Ebner, Well Le Locle, Switzerland). Two sections of approximately 400 μm thickness were removed from this specimen as a control section. The remaining portion was cut into two experimental samples of approximately 4×2 mm.

The experimental and control lesions were sterilized by gamma irradiation with a dose of 70–80 Grays/hour over 48 hours, i.e. 3360/3840 total exposure under a $Cobalt^{60}$ source. Amaechi *et al.* (1999) have established that this dose sterilizes an enamel specimen of bacteria, without causing discolouration or change in mineral loss values. A recent *in vivo* study (Kielbassa *et al.*, 2000) has confirmed that irradiation does not have a significant effect on the de/remineralization potential of an *in situ* specimen, although this was a much lower dose of radiation than that advocated by Amaechi *et al.* (1999).

The control sections were prepared prior to placement of the enamel specimens in the mouth

to ensure that there was a suitable subsurface lesion. The sections were ground using a manual diamond grinding plate with distilled water irrigation to approximately 100 μm thickness, turning them once to ensure that they were planoparallel. The thickness of the sections was checked using a micrometer (Mitutoyo Digimatic, Mitutoyo Corporation, Kawasaki-Shi, Japan). The sections were examined using polarized light microscopy for a subsurface lesion of even quality. Specimens with evidence of surface lesions or lesions of poor quality were rejected. The control sections from each acceptable lesion were placed together with an aluminium step-wedge with 25 μm steps, on high-resolution radiographic plates (Microchrome Technology Inc., San Jose, CA, USA). They were radiographed in a Phillips X-ray set with a copper target and nickel filter. The exposure time was 18 minutes at 25 kV and 10 mA. The anode film distance was 30 cm.

The microradiograph images were developed. They were scanned perpendicular to the enamel surface under standard light intensity and magnification using an optical microscope (Leica Microsystems Inc., Wetzlar, Germany) equipped with a CCD camera (Sony model XC-75CE, Sony Corp., Japan). Suitable images were captured and measurement of the lesion parameters carried out on a computerized image analysis system (TMRW program version 1.22, Inspektor Research Systems BV, Amsterdam, The Netherlands) using an algorithm developed by de Josselin de Jong *et al.* (1987). The mineral content of the sections was expressed as mineral loss (ΔZ), lesion depth (l_d), lesion width (l_w), and ratio ($\Delta Z/l_d$; Figure 1).

The experimental enamel specimens were mounted onto customized holders (Figure 2) using a dentine and enamel primer with a light-cured composite resin (Prime and Bond/Prismafil, Dentsply De-Trey, Konstanz, Germany).

The amount of fluoride released from the elastomeric ligatures was tested by immersing three ligatures in a polyethylene bottle containing artificial saliva. The concentration of fluoride after 24 hours was measured using the potentiometric analytical method. The mean cumulative level of fluoride released was calculated to be

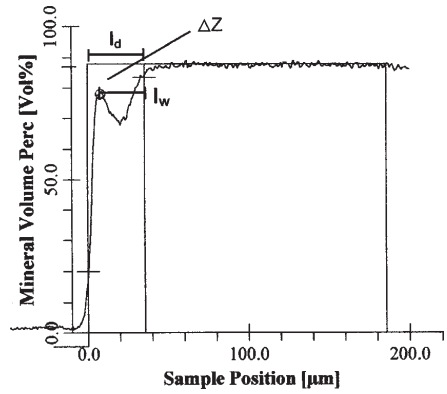

Figure 1 Diagram of a transverse microradiography plot showing the measurements used to calculate the parameters of mineral loss (ΔZ), lesion depth (l_d), and lesion width (l_w).

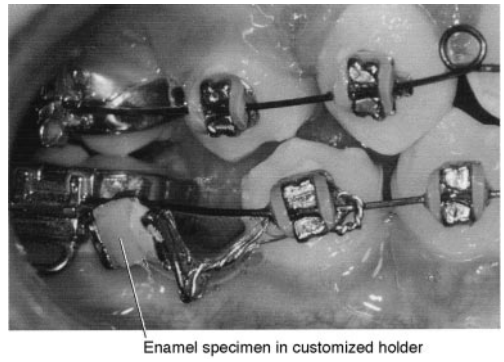

Figure 2 Clinical photograph of the enamel specimen in the customized holder.

0.9 μg/ml/elastomeric over 24 hours, which was similar to that reported by Wiltshire (1996) of 1.3 μg/ml/elastomeric over 24 hours. The lower level of fluoride release may be accounted for by the use of artificial saliva being used as the storage medium rather than distilled water (Levallois *et al.*, 1998).

Subjects and clinical method

Data from a previous experiment (Benson *et al.*, 1999) were used to perform a sample size calculation. This showed that using a crossover design, a sample size of 14 would provide an 85 per cent probability of detecting a difference in mineral loss at the 0.01 per cent level.

Approval was obtained from the local ethics committee. The subjects for the trial were drawn from individuals about to start fixed orthodontic treatment at Liverpool University Dental Hospital, who required extractions as part of their orthodontic treatment plan. Informed consent was obtained. Clinical procedures were carried out as follows:

Visit 1 (placement of the appliance). The patient was randomly allocated, by a block randomization technique, to either receiving the fluoridated elastomeric ligatures (Force A Ligature Ties, A-Company, San Diego, CA, USA) or the non-fluoridated grey ligatures (American Orthodontics, Sheboyan, WI, USA) at the first visit. Following placement of the bands and brackets, the customized holder with the *in situ* enamel specimen was placed on the archwire (Figure 2). Patients were supplied with standardized toothpaste (Aquafresh, Smith Kline Beecham Consumer Healthcare, Brentford, UK, total fluoride 1055 ppm) and asked to refrain from any other oral hygiene products for the duration of the trial. The patient was given an appointment 4–6 weeks later to adjust the appliance.

Visit 2 (first adjustment appointment). The customized holder with the enamel specimen was removed. Non-fluoridated elastomeric ligatures were placed to allow a washout period before the second phase of the experiment. An appointment was made for 4–6 weeks later for the second adjustment.

Visit 3 (second adjustment appointment). A second customized holder with an *in situ* enamel specimen was placed on the archwire. The elastomeric ligatures used to secure the archwire were either non-fluoridated, if fluoridated ligatures were used at the first visit, or *vice versa*. More toothpaste was supplied if required. The patient was given an appointment to adjust the appliance in 4–6 weeks.

Visit 4 (third adjustment appointment). The second customized holder with enamel specimen was removed.

Sixteen individuals were approached to take part in the trial. One refused due to concerns about fluoride and one because they considered the holder to be uncomfortable. Of the 14 patients recruited for the trial, one patient was excluded due to an error with the ligatures causing them to receive the non-fluoridated ligatures, instead of the fluoridated ligatures. One specimen was lost due to fracture of the archwire; none was lost during processing. Therefore, a total of 24 enamel specimens were analysed from 12 patients.

The mean age of the patients was 14.8 years (range 13.3–17.7), and there were seven males and five females. The average number of ligatures placed for each patient was 15 (range 12–20). The average time the fluoridated ligatures were in place was 43 days (range 31–70), the non-fluoridated for 42 days (range 20–55), and the average washout period was 51 days (range 29–73).

Measurement of de/remineralization

Following removal from the mouth, the specimens were separated from the customized holders with an orthodontic debonding instrument. The enamel was stored in distilled water, before being prepared for microradiography. The specimens were prepared in the same way as the control specimens. They were cut perpendicular to the surface and polished to give several plano-parallel sections of approximately 100 μm thickness. The majority of specimens were cut into two to four sections; however, three specimens were divided into six sections. After preparation, the specimens were recoded by a second investigator to allow blind assessment by the principal investigator.

Therefore, for each patient there were several enamel sections from the two experimental enamel specimens and the two control sections. All the sections from one patient were microradiographed on the same plate, together with the calibrating step-wedge. The control sections were microradiographed a second time with all the experimental sections, to minimize error due to problems with exposure and developing. The microradiographs were quantified as previously described by computerized image analysis.

Statistical analysis

Each specimen was divided to provide between two and six sections depending on the size of the original specimen. All the sections were examined and a total of between two and five readings were taken to obtain a representative reading for that specimen. The mean of these readings was then chosen for statistical analysis. All statistical tests were carried out using SPSS for Windows version 8 (SPSS Inc., Chicago, IL, USA).

The null hypothesis was that there was no difference between the lesion parameters of the enamel specimens used for the control, fluoride, and non-fluoride ligatures. The data were examined graphically and tested with the Shapiro–Wilk statistic, which confirmed it to be normally distributed; therefore, parametric statistics were applied. Hypothesis testing was carried out with a one-factor within subjects' analysis of variance.

Results

The descriptive statistics of the four parameters measured for the control, non-fluoridated, and fluoridated specimens are given in Table 1. These show that the means for the parameters measuring the size of the lesion were increased for the two specimens that were placed in the mouth, compared with the control that had not been in the mouth. The range and variability were also greater. The means and variability were slightly lower for the fluoride compared with the non-fluoride specimens.

The results of the one-factor within subjects ANOVA (Table 2) showed no differences between the groups.

Table 3 shows the number of enamel specimens placed with the non-fluoride and fluoride ligatures that either gained or lost mineral compared with the control specimen after a period in the mouth. There were similar numbers of fluoride and non-fluoride ligature specimens that gained or lost mineral. In two individuals both specimens gained mineral compared with the control and in five individuals both specimens lost further mineral. In seven individuals, the mineral loss was greater in the non-fluoride specimens compared with the fluoride specimens and in five individuals the mineral loss was greater in the fluoride specimens.

Table 1 Means, standard deviations, confidence intervals (CI), and ranges for control, non-fluoridated, and fluoridated specimen parameters of mineral loss (vol %.μm), lesion width (μm), lesion depth (μm), and percentage mineral loss (vol %).

Parameter	Statistic	Control	Non-fluoridated	Fluoridated
Mineral loss (vol %.μm)	Mean	403.7	599.3	477.2
	SD	139.5	515.4	298.4
	95% CI	315.0–492.3	271.8–926.7	287.6–666.8
	Min	240.9	116.6	116.6
	Max	755.3	2071.1	1194.7
Lesion depth (μm)	Mean	39.9	48.0	45.1
	SD	4.5	18.7	15.0
	95% CI	37.0–42.7	36.1–59.9	35.6–54.6
	Min	27.5	23.6	23.6
	Max	44.8	91.0	71.3
Lesion width (μm)	Mean	30.3	36.8	34.2
	SD	3.3	19.6	13.6
	95% CI	28.2–32.4	24.4–49.3	25.6–42.9
	Min	23.2	13.1	13.1
	Max	33.6	83.1	59.9
Ratio (vol %)	Mean	10.1	11.5	9.8
	SD	3.0	5.5	2.6
	95% CI	8.2–12.0	8.0–15.0	8.1–11.4
	Min	6.6	5.2	5.9
	Max	17.2	22.8	15.8

Table 2 The results of the one-factor within subjects ANOVA carried out between the specimens that were used for the control, and those placed with the non-fluoridated and fluoridated ligature parameters of mineral loss (vol %.μm), lesion width (μm), lesion depth (μm), and percentage mineral loss (vol %).

Parameter	*F*-statistic	*P*
Mineral loss (vol %.μm)	1.02	0.376
Lesion depth (μm)	1.06	0.365
Lesion width (μm)	0.63	0.540
Ratio (vol %)	0.74	0.489

P is the probability that the null hypothesis is true.

Table 3 The number of enamel specimens placed with the non-fluoride and fluoride ligatures that either gained or lost mineral compared with the control specimen after a length of time in the mouth (mean 43 days for fluoridated and 42 days for non-fluoridated ligatures).

	Non-fluoride ligature specimens*	Fluoride ligature specimens
Mineral loss decreased	3	5
Mineral loss increased	8	7

*One specimen had the same mineral loss.

Discussion

The aim of this study was to use the *in situ* caries model to test the effect of fluoride- and non-fluoride-releasing elastomeric ligatures on enamel specimens with an early artificial carious lesion. The results show that there was no statistically significant difference in the parameters of the carious lesions between either those enamel specimens that had been placed with fluoride- and non-fluoride-releasing ligatures, or the control that had not been placed in the mouth. This suggests that fluoride-releasing ligatures do not provide a significant anti-cariogenic benefit in orthodontic patients. However, the results of this study do not preclude the possibility that fluoride-releasing ligatures have a significant local effect around the bracket.

This local effect was investigated by Wilson and Love (1995), who used an *in vivo* model to investigate fluoride-releasing elastomerics on enamel directly cervical to an orthodontic bracket. They studied four patients, each with four premolars destined for extraction during orthodontic treatment. Orthodontic brackets were placed on these teeth, with fluoride-releasing ligatures over the brackets on one side of the mouth and conventional elastomeric ligatures over the brackets on the contralateral side. After four weeks the teeth were extracted, sectioned, and analysed using microhardness testing. The enamel was found to be significantly harder at a depth of 20 μm in the teeth with the fluoridated ligatures, compared with the teeth with non-fluoridated ligatures.

The local effect of ligatures would be difficult to investigate using the *in situ* model, because of the small nature of the enamel specimen. Whilst the *in vivo* model has the advantage of studying demineralization in whole, vital teeth, it has the disadvantage of not being able to record the baseline mineral content of the enamel. O'Reilly and Featherstone (1987) pointed out that variation in tooth enamel within a person can be as great or greater than variation between individuals. The method of cross-sectional microhardness employed by Wilson and Love (1995) does provide an indirect assessment of demineralization and remineralization, but Arends and ten Bosch (1992) state that the results for the outer 25 μm cannot be used. Transverse microradiography is recognized as the most practical technique for the direct and quantitative measurement of mineral content and changes (Arends and ten Bosch, 1992).

The mean mineral loss in the experimental enamel specimens in this study was higher than the controls. This suggests that the enamel was subject to further demineralization in the mouth, although this was not statistically significant. This is contrary to the results of Benson *et al.* (1999), who found that an enamel specimen without an orthodontic bracket base remineralized significantly more than a specimen with a small bracket base placed on the surface. The probable explanation for this is that the mineral loss values at the start of the previous experiment

(Benson *et al*., 1999) were higher than during this experiment. It has been shown (Strang *et al*., 1987) that the baseline lesion mineral loss values have a direct relationship on remineralization rates. Therefore, the higher baseline mineral loss values in the work by Benson *et al*. (1999) would encourage remineralization. Lower initial mineral loss values were used in this experiment because it was considered that the effect of the ligatures on demineralization, as well as remineralization, needed to be examined.

A bracket base was not placed on the specimens in this study. Those used by Benson *et al*. (1999) were too small to accommodate a ligature. It was therefore decided to examine the effects of the ligatures on enamel de/remineralization in the mouth as a whole. Work is currently being carried out to design a bracket that may be placed on an enamel specimen to simulate the local effect of the fluoridated ligature.

The benefit of the fluoride ligatures may be short-term. Wiltshire (1996) found that *in vitro* there was an initial burst of fluoride discharged on the first day, which then decreased logarithmically. Wilson and Gregory (1995) showed that the proportion of *Streptococcus mutans* in the total salivary streptococci population fell when measured one week after placement of fluoride-releasing ligatures, but returned to baseline levels at two weeks.

It has been speculated that elastomeric ligatures in the mouth absorb fluoride as well as releasing it (Wiltshire, 1999). That author found that more fluoride was released from ligatures when they had been in the mouth for one month, compared with ligatures stored in distilled water. This occurred with both fluoridated and non-fluoridated ligatures, but the effect was greater with the fluoridated ligatures. This fluoride recharge may be of significant clinical benefit.

Several authors have observed that fluoride-releasing ligatures deteriorate rapidly in the mouth, becoming enlarged and discoloured (Miethke, 1997; Wiltshire, 1999; Banks *et al*., 2000) and it has been suggested that the properties of the elastomeric materials are adversely affected by the addition of fluoride (Miethke, 1997). The type of ligature used in this investigation did not display any major deterioration in the mouth.

The concern of the patient who declined to enter the trial due to the fluoride content of these elastics is likely to be unfounded. Wiltshire (1996) found that the maximum rate of fluoride release was in the first 24 hours, when 20 ligatures released an average of 0.026 mg of fluoride. The amount of fluoride released is likely to be lower in the mouth as it has been shown that fluoride release from glass ionomer cements is lower in saliva compared with distilled water (Damen *et al*., 1996). Barnhart *et al*. (1974) found that a group of 11–13-year-olds ingested a mean value of 0.07 g of toothpaste per brushing. Assuming that they brushed twice a day with toothpaste containing 1000 ppm fluoride, this would amount to an ingestion of fluoride from toothpaste alone of 0.14 mg. This is much more than the fluoride released from the ligatures during the first 24 hours, when fluoride release is at a maximum.

Conclusions

No overall anti-cariogenic benefit from using fluoride-releasing elastomeric ligatures in orthodontic patients was detected using the *in situ* caries model. The local effects of the ligatures require further investigation.

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