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SCIENTIFIC SECTION

Fluoridated elastomers: *in vivo* versus *in vitro* fluoride release

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Abstract

Objectives: To compare (i) the *in vivo* release of fluoride from fluoridated elastomers to the *in vitro* release, and (ii) the residual fluoride content of the elastomers after 1 week in the mouth with and without fluoride toothpaste and mouthrinse.

Design: A prospective, longitudinal, cross-over study.

Subjects and method: Six subjects were recruited by poster to take part in the study. Each subject had one premolar in each quadrant to which a bracket could be fixed and exemplary oral hygiene. Elastomers were then placed on these brackets.

Intervention: The study was divided into two parts: (i) subjects used oral hygiene products with fluoride and (ii) oral hygiene products with fluoride were excluded. Both groups of elastomers were left in the mouth for 1 week. After collection the elastomers were stored in distilled water.

Main outcome measures: The amount of residual fluoride in the ligatures after they have been placed in the mouth for 1 week was compared with the cumulative fluoride release *in vitro* over 1 week and 6 months.

Results: Only 13 per cent of the total amount of fluoride in fluoridated elastomers was released during the first week *in vitro*, compared with 90 per cent *in vivo*. There was a significantly greater amount ($P = 0.001$) of residual fluoride when the elastomers were in the mouth for 1 week in the presence of fluoride toothpaste and mouthrinse, than when fluoride supplements were excluded.

Conclusions: (1) Higher levels of fluoride are lost from the fluoride elastomers *in vivo* than *in vitro* during the first week. (2) A significantly greater amount of residual fluoride was released from the elastomers placed in the mouth when fluoride toothpaste and mouthrinse were used.

Index words: Demineralization, fluoride, elastomer, orthodontic.

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Introduction

Fluoride supplements have been shown to reduce the incidence of demineralization during orthodontic treatment.[1,2] One problem with these methods of administrating fluoride is that they are dependent on patient compliance.

Recently, elastomers that release stannous fluoride have been made available for orthodontic patients. The elastomers leach fluoride close to the site of vulnerability next to the bracket. Because they are changed every 4–6 weeks, they may be the perfect vehicle to provide a low concentration of fluoride over a period of orthodontic treatment. Clinical studies using these elastomers have shown promising results with a reduction in the prevalence[3] and severity[4] of demineralization during orthodontic treatment. *In vitro* studies, however, have shown that although the fluoride release is initially high it soon becomes low and not sustainable over clinically relevant time periods.[5]

To-date few studies have examined the *in vivo* fluoride release from fluoridated ligatures. Wiltshire[6] has suggested that the fluoridated elastomers absorb fluoride in the mouth, as well as release it. This has also been demonstrated with some of the dental cements.[7] It would obviously be difficult to directly measure the amount of fluoride released or absorbed from the elastomers *in vivo*. However, by measuring the amount of fluoride left in the

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elastomers after they have been in the mouth for a period of time and comparing this with the total amount of fluoride released from the elastomers *in vitro*, it should be possible to provide an indirect estimate of the amount of fluoride released. Further to this, the *in vitro* part of the study will also allow comparisons to be made between the fluoride release into a test tube to that in the mouth.

The aims of this *in vivo* study were:

- to compare the amount of fluoride released from fluoridated elastomers placed in the mouth for 7 days with similar elastomers placed in distilled water;
- compare the amount of residual fluoride released from fluoridated elastomers placed in the mouth for 7 days when fluoride supplements were used with similar elastomers placed without the use of fluoride supplement.

Materials and methods

Ethical approval was obtained from the South Sheffield Research Ethics Committee. They made the proviso that a poster should be used to recruit volunteers. A sample size calculation showed that five individuals were needed to show a difference of 0.56 μgF/ml/elastomer, to a power of 0.95 and significance of 0.01.

The study group

Six subjects (three males, three females) were recruited to take part in the study. Subjects were eligible for inclusion if they had at least one premolar in each quadrant, to which a bracket could be fixed and their oral hygiene was of an exemplary standard. For all the subjects that were eligible for inclusion, the trial was explained and each received a patient information sheet. At the next appointment written consent was obtained.

The intervention

Each subject had an orthodontic bracket (GAC international. Inc, 185 Oval Drive, Islanda, NY 11749) placed onto a premolar in each quadrant (Figure 1). Standard methods and materials were used to place the bracket, including the use of 30 per cent phosphoric etchant gel to treat the surface of the premolar. They were attached with a non-fluoride leaching composite bonding cement (Relya –Bond®, Reliance Ortho Prod. Inc., Itasca, IL, USA). Following bracket placement the subjects were given oral hygiene instruction. All subjects were asked to maintain good oral hygiene for the duration of the study.

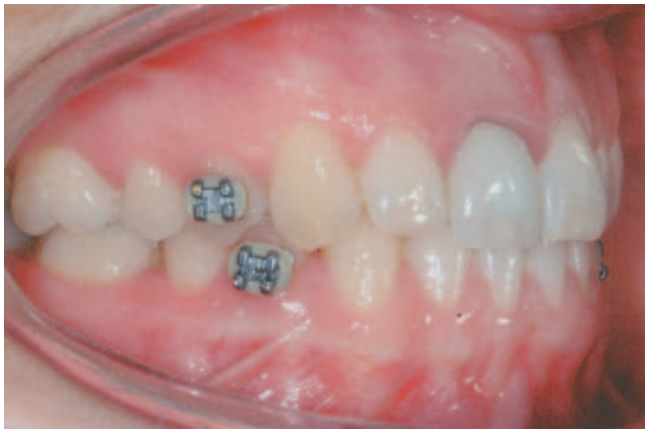

Fig. 1 Brackets positioned on the premolars, with fluoridated elastomers in place.

The in vivo *study with fluoride supplements.* One fluoridated elastomer (Fluor-I-Ties®, Ortho Arch Company Inc) was tied onto each of the four brackets and left in the mouth for 7 days. During this period, the subjects were asked to use a standardized fluoridated mouthwash (Colgate FluoriGard®, Sodium fluoride 0.05 per cent, Colgate Palmolive (UK) Manchester M5 3FS) and toothpaste (Colgate Fluoride toothpaste, Sodium monofluorophosphate 1000 ppm, sodium fluoride 450 ppm). The subjects were asked to use these products at set times of the day, namely 7.30 a.m. and 7.30 p.m.

The elastomers were removed after 7 days, then rinsed with distilled water to remove debris and protein accumulations. The four ties from each volunteer were placed collectively into an air-tight, polyethelene beaker containing 10 ml of distilled water and stored at room temperature.

The in vivo *study without fluoride supplements.* The method outlined above was repeated, except for the 7-days that the elastomers were left in the mouth the subjects were prescribed a non-fluoridated mouthwash and toothpaste (Sainsbury's Confident Naturally Fresh, Stamford Street, London SE11 9LL). Subjects were also asked to avoid other fluoride supplements for the duration of the study.

Fluoride analysis

In order to calculate the fluoride concentrations of the distilled water a fluoride/fluoride combination electrode (Thermo Orion) was used. The electrode was connected to a pH/mV with readability to 0.1 mV. Before analysing the samples the electrode was calibrated, using two stand-

ard solutions of fluoride (1 and 10 ppm). To maintain a standard pH all solutions were mixed with equal amounts of total ionic solution adjustment buffer (TISAB II). Calibration of the electrode was repeated every 2 hours to ensure the readings were accurate and consistent.

The in vivo *elastomers*. Fluoride analysis was carried out incrementally (approximately every 6 weeks) over 6 months until most of the fluoride had been released. Six weeks was considered to be a sufficiently long time period to allow fluoride to leach out of the elastomers without allowing super saturation of the solutions. At each of the analysing sessions the four elastomers were removed from each sample and transferred to a fresh beaker containing 10 ml of distilled water. The new sample was then stored for a further 6 weeks in the laboratory before analysis. A 1-ml pipette was used to draw equal quantities of the solution under test (taken from the original beaker) and TISAB II, which were mixed together in a disposable container. This solution was then analysed with the fluoride electrode and once the voltmeter had stabilized, the reading was recorded. Between readings the electrode was carefully cleaned with distilled water and dried with a tissue. At the completion of each session the electrode was cleaned and stored according to the manufacturers instructions.

The in vitro *elastomers*. Four elastomers were placed onto orthodontic brackets, with 1 ml of distilled water in a polyethelene beaker and stored in an incubator at 37°C. The fluoride/fluoride combination electrode was used to analyse the fluoride content of the distilled water after 24 hours. The same four elastomers were placed in a fresh 1-ml sample of distilled water and again stored for 24 hours. The fluoride concentration of the distilled water was again measured. Daily measurements of fluoride release were carried out during the first week. This was followed by incremental measurements over the next 6 months until it was considered that most of the fluoride had been released from the elastomers.

Throughout the testing period of the study a distilled water control was used. This was obtained from the same source as the water used as the storage medium for the elastomers. The same conditions were applied to the control as to the solutions under test.

Statistical testing

The following statistics were employed in this study:

1. The total amount of fluoride released *in vitro* was calculated by adding the areas under the curve (AUC) between each pair of observations as follows. If we have fluoride concentrations y_1 and y_2 at times t_1 and t_2, the AUC between those two times is the product of the time difference and the average of the two measurements. Thus, we get:

$$(t_2 - t_1)\,(y_1 + y_2)/2$$

This is known as the trapezium rule because of the shape of each segment of the area of the curve. The total amount of fluoride left in the *in vivo* elastomers was calculated by adding the incremental fluoride measurements taken over the 6 months.

2. To test the null hypothesis that there was no difference between the fluoride released from the elastomers worn during the fluoride supplement period compared with the non-fluoride supplement period, a paired *t*-test was used. Prior to testing, the data was examined for normality using the Shapiro–Wilks test.

Results

The mean total fluoride concentration per module *in vitro* over the 6-months for each of the ten groups is shown in Table 1. The mean area under the curve for the 10 samples was 138.65 μgF/day/ml/elastomer (SD 3.75). The mean total amount of fluoride available for release from each ligature was therefore 0.139 mg. The *in vitro* mean cumulative 7-day fluoride concentration per module was 18.07 μgF/ml/elastomer. The mean total amount of

Table 1 The cumulative fluoride concentration per elastomer (μgF/day/ml/elastomer) from 10 *in vitro* samples of fluoridated elastomers over 6 months calculated using the area under the curve (AUC) method

Sample number	AUC
1	140.26
2	139.14
3	130.91
4	138.55
5	137.82
6	135.39
7	144.17
8	139.64
9	141.95
10	142.01
Mean	138.65
SD	3.75
95% CI	136.30–141.67

fluoride released from each ligature during the first 7 days *in vitro* was therefore 0.018 mgF. Hence, 13 per cent of the available fluoride was released during the first week *in vitro*.

The cumulative residual fluoride concentration from the elastomers that had been placed in the mouth is shown in Table 2. The mean cumulative residual fluoride concentration was 13.30 μgF/ml/elastomer (SD 0.74) for the fluoride elastomers worn without fluoride supplements and 15.85 μgF/ml/elastomer (SD 0.72) for those worn with fluoride supplements. The mean total amount of residual fluoride in each elastomer was therefore 0.013 mg for the fluoride elastomers worn without fluoride supplements and 0.016 mg for those worn with fluoride supplements.

To calculate the amount of fluoride lost in the mouth during the first week a subtraction method was used. The mean total amount of residual fluoride from the *in vivo* part of the study was subtracted from the mean total amount of fluoride from the *in vitro* part of the study. The mean total amount of fluoride in each elastomer calculated *in vitro* was 0.139 mg. The mean residual amount of fluoride per elastomer was 0.013 mg for the fluoride elastomers worn without fluoride supplements. The difference in these amounts is very large, and suggests that over 0.12 mg of fluoride or 90 per cent of the available fluoride was lost per module, in the mouth of each volunteer during the first week.

Throughout the study a control was used. This was distilled water obtained from the same source as that used for the fluoride analysis. The fluoride levels remained constant and extremely low (<0.02 μgF/ml) for the whole study indicating that the increase in fluoride was coming solely from the elastomers and not from any other source (i.e. the beaker).

At the end of the 6-month experimental period both *in vivo* and *in vitro* samples were still releasing a low level of fluoride. To ensure that the amount of residual fluoride was the same for both the *in vivo* and *in vitro* elastomers the samples were placed in fresh distilled water and the fluoride concentration was tested after 24 hours. The fluoride release was found to be the same for both the *in vivo* (0.41 μgF/ml/elastomer) and *in vitro* groups (0.41 μgF/ml/elastomer). Therefore, as the amount was small and similar in both groups it was considered possible to compare the results from the two groups.

The effect of fluoride supplementation on the in vivo modules

To test the hypothesis that there was a difference in the residual fluoride concentration between the *in vivo* elastomers collected with fluoride supplements compared with the elastomers collected without fluoride supplements the data were first checked for normality using the Shapiro–Wilks test. This test was non-significant thus allowing the paired *t*-test to be utilized. The mean difference, standard deviation and 95 per cent confidence limits of the differences between the two groups are shown in Table 3. The significance level ($P = 0.001$) indicates very strong evidence that there is a difference between the residual fluoride levels in the two groups.

Discussion

The results of this study suggest that fluoride is released from fluoridated elastomers more rapidly when placed in the mouth compared with in the test tube. The amount of fluoride released from each elastomer *in vivo* was approximately 0.12 mg. If the average orthodontic

Table 2 Cumulative residual fluoride concentration per module (μgF/ml/elastomer) measured over 6 months for fluoridated elastomers worn by six individuals for 1 week, with and without fluoride mouthwash and toothpaste

Subject	Without fluoride supplements	With fluoride supplements
1	13.44	16.23
2	14.12	16.43
3	12.96	15.83
4	12.00	15.73
5	13.71	16.38
6	13.58	14.50
Mean	13.30	15.85
SD	0.74	0.72
95% CI	13.25–14.12	14.76–15.54

Table 3 Mean difference, standard deviation, 95 per cent CI and results of paired *t*-test for the cumulative residual fluoride concentration per elastomer (μgF/ml/elastomer) measured over 6 months from the fluoridated elastomers worn by six individuals for 1 week, with and without fluoride toothpaste and mouthrinse

Paired differences	Mean difference	–2.55
	Standard deviation	0.93
	Standard error of the mean	0.38
95% CI	Lower	–3.52
	Upper	–1.58
	Significance	0.001

patient has 20 modules placed at each appointment, 2.4 mg of fluoride is released during the first week in the mouth. It has been suggested that the daily intake of fluoride in children should not exceed 0.10 mg/kg of body weight.[8] If the release of fluoride quickly drops off after the first few days, the fluoride release may be extremely high immediately after the elastomers are placed. Caution is warranted when using these modules, particularly for younger patients, those with an already high fluoride intake from other sources, such as fluoride supplements or in areas where the water is fluoridated. One solution to reduce this initial high dose of fluoride is to place them only on the teeth at risk of demineralization or alternate them with regular modules. Further work is required to investigate fluoride loss from these elastomers in the first 24 hours after placement.

Another finding of this investigation was that there is a significant difference in the amount of residual fluoride in the modules when fluoride supplements were used compared with when they were avoided by the subjects. This confirms work carried out previously,[6] which suggested that the modules not only release fluoride, but also absorb it from their environment. One criticism of the fluoridated elastomers is that their ability to release fluoride is not sustainable over time.[5] If the modules are able to recharge with fluoride introduced into the environment from toothpastes or mouthwashes, then sufficient amounts of fluoride might be released over longer time periods to prevent demineralization. Several factors are likely to be involved in this recharge,[7] including the permeability of the material, and the form and concentration of the fluoride used; however, this study appears to suggest that the elastomers do have a recharge potential. The clinical significance of this recharge potential is speculative. The patients who are susceptible to demineralization are likely to be non-compliant having an irregular intake of fluoride from toothpastes or mouthwashes. Ideally, there should be sufficient amounts of fluoride released from the elastomers over clinically relevant time periods to prevent demineralization without relying on fluoride recharge.

The pattern of fluoride release in the *in vitro* part of this study was similar to that reported by Wiltshire.[5] However, the amount of fluoride released at each time interval in this study was much higher.

One explanation for this finding is that the elastomers may not be the same in both studies. The appearance of the elastomers certainly has changed; instead of being injection moulded they are now cut from a tube. This is illustrated in Figure 2.

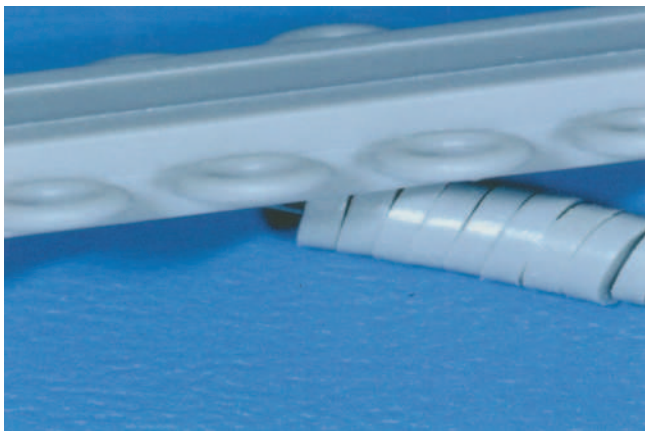

Fig. 2 Recent change in physical appearance of the elastomers.

It is likely that not only has the appearance of the modules changed since Wiltshire's work, but so have their physical and chemical properties. Several clinicians have found that the original fluoridated elastomers performed poorly in the mouth.[3,9] They reported high breakages and unacceptable swelling of the modules. Wiltshire[6] found that they swelled and doubled in weight after 4 weeks in the mouth, whereas little change was measured with conventional elastomers. It is possible that the manufacturer has addressed these problems and changed the elastomers. It would appear that elastomers produced by a die punching technique are 50–80 per cent stronger than those produced by injection moulding.[10] From their appearance the elastomers have changed from injection moulded to die punched.

It was found that, even after 6 months, the elastomers were still releasing fluoride; therefore, our calculation of the total amount of fluoride available for release from each elastomer might be an underestimation. This is possibly because there is an outer, loosely bound layer of fluoride that is released initially and an inner, more tightly bound core of fluoride that is released more slowly. However, because the 24-hour release from the *in vivo* and *in vitro* elastomers was the same after 196 days, there is likely to be a similar amount of fluoride left in both groups of elastomers; therefore, the proportion of fluoride released after 7 days in the mouth will not be altered.

Although 90 per cent of the fluoride is released during the first week in the mouth, the remaining 10 per cent might be released more slowly and could be sufficient to reduce the prevalence and severity of demineralization.[3,4] Margolis *et al.*[11] found that concentrations of fluoride as low as 0.024 ppm offered 'remarkable protection' of the

enamel surface *in vitro*. If this is true *in vivo*, then it is likely that fluoridated elastomers, particularly combined with fluoride toothpaste and mouthrinse, will raise the fluoride level in plaque sufficiently to enhance remineralization. This would be a useful area of further investigation.

This study had a small sample size, but one surprising finding was that the variability in the amount of residual fluoride left in the elastomers after 1 week was low between individuals. This suggests that the fluoride release from these modules is consistent regardless of differences in diet, flow of saliva and fluoride clearance patterns. Because this variability was low, the power of the study was sufficient to show a positive difference in the residual fluoride when fluoride supplements were used.

This study explored fluoride release from fluoridated elastomers after 1 week in the mouth. Further investigations leaving them in place in the mouth for 2, 3, 4, and 6 weeks are warranted. This would enable the rate of fluoride release to be more accurately profiled and help ascertain the optimal period for replacing fluoridated elastomers in the clinical situation. It would also be interesting to determine if non-fluoridated elastomers have the same recharge potential.

Conclusions

1. In the first week after placement, fluoridated elastomers release high levels of fluoride.
2. The *in vitro* and *in vivo* fluoride release does not appear to be similar.
3. There was a significantly greater amount ($P = 0.001$) of residual fluoride when the elastomers were in the mouth for 1 week in the presence of fluoride containing hygiene products than when they were excluded.
4. Fluoridated elastomers may imbibe fluoride from their environment.

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