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Fluoridated Elastomers – Effect on the Microbiology of Plaque

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Abstract

Objective: To investigate the effect of fluoridated elastomeric ligatures on the microbiology of the local dental plaque *in vivo.*

Design: A randomised, prospective, longitudinal clinical trial, employing a split mouth, crossover design.

Sample population and setting: 30 individuals at the beginning of their treatment with fixed orthodontic appliances in the Orthodontic Departments of Liverpool and Sheffield Dental Hospitals.

Method: The study consisted of two experimental periods of six weeks with a washout period between. Fluoridated elastomers were randomly allocated at the first visit to be placed around brackets on 12, 11, 33; or 22, 21, 43. Non-fluoridated elastomers were placed on the contra-lateral teeth. A standard non-antibacterial fluoridated toothpaste and mouthwash were supplied. After 6 weeks (visit 2) the elastomers were removed, placed in transport media and plated on agar within two hours. Non-fluoridated elastomers were placed on all brackets for one visit to allow for a washout period. At visit 3 fluoridated elastomers were placed on the contra-lateral teeth to visit 1. At visit 4 the procedures at visit 2 were repeated. Therefore the experiment was performed over a total of four treatment visits, with samples collected on visit 2 and 4. A logistic regression was performed with the dependent variable being the presence or absence of streptococcal or anaerobic growth. A mixed effects ANOVA was carried out with the dependent variable being the percent streptococcal or anaerobic bacterial count.

Results: The only significant independent variables were the subject variable ($p=<0.001$) for the percent streptococcal and anaerobic bacterial count and the visit variable for the percent streptococcal count ($p=<0.001$). The use of fluoridated or non-fluoridated elastomers was not significant for either percent streptococcal count ($p=0.288$) or anaerobic count ($p=0.230$).

Conclusion: Fluoridated elastomers are not effective at reducing local streptococcal or anaerobic bacterial growth after a clinical relevant time in the mouth.

Introduction

Plaque is a major aetiological factor in the development of dental caries. The control of plaque is fundamental in the control of caries and periodontitis. It has been shown that the placement of a fixed orthodontic appliance leads to both an increase in the levels[1] and a change in the composition of dental plaque[2]. Sakamaki and Bahn[3] showed an increase in lactobacillus index and salivary lactobacillus counts with the placement of orthodontic bands. Corbett *et al*[4] demonstrated an increase in the level of *Streptococcus mutans* in the plaque surrounding an orthodontic appliance. Scheie *et al*[5] demonstrated an increase in the levels of *Streptococcus mutans* in saliva and suggested that the placement of an orthodontic appliance leads to the creation of new retentive areas favouring the local growth of this organism.

Elastomeric ligatures that release stannous fluoride are commercially available[6]. Maltz and Emilson[7] showed that stannous and cupric fluoride had a superior antimicrobial effect on streptococci and lactobacilli over sodium or ammonium fluoride and suggested that the metal ions play a large part in their bactericidal effect. A reduction in the level of *Streptococcus mutans* in the saliva with these elastomers has been demonstrated[8]. If fluoridated elastomers are shown to adversely affect local cariogenic bacteria, they will be important in the reduction of enamel demineralisation around orthodontic brackets.

The objective of this study was to investigate the effect of fluoridated elastomeric ligatures on the bacterial count of dental plaque forming on the ligatures *in vivo.*

Materials and Methods

This was a prospective, randomised clinical trial, employing a split mouth, crossover design. Volunteers were recruited from patients about to start their orthodontic treatment with upper and lower fixed appliances in the orthodontic departments of Liverpool University Dental Hospital and the Charles Clifford Dental Hospital, Sheffield. Patients who were pregnant; diabetic; using an antimicrobial mouthwash; using any complicating medicine or patients with a history of antibiotic use in the last two months were excluded. All patients visited a hygienist who provided standardized oral hygiene instruction. They had to achieve a score of 1 on the Index of Oral Cleanliness prior to placement of fixed appliances

Ethical approval was obtained from the Local Research Ethics Committees. Eligible patients were invited to participate in the study at a visit before the fixed appliances were placed. Informed, written consent was given by the patients and their parents agreeing to enter the study on the visit the appliances were placed. This was usually two weeks after the initial discussion.

The following procedures were carried out:

Visit 1: The fixed appliance brackets and bands were placed. The patients were randomly allocated to having the fluoridated elastomers (Fluor-I-Ties, Ortho Arch Co., Schaumburg, IL USA) either on the upper left lateral incisor, upper left central incisor and lower right canine; or the upper right lateral incisor, upper right central incisor and lower left canine. The randomisation was carried out using computer generated random numbers in a block design. The allocation was concealed in consecutively

numbered, sealed, opaque envelopes. Conventional non-fluoridated elastomers were placed on the remaining teeth. The patients were provided with a standard fluoridated toothpaste (Aquafresh; monofluorophosphate 0.75% w/w and sodium fluoride 0.01% w/w; total fluoride 1,055ppm SmithKline Beecham Consumer Healthcare, Middlesex, UK.), with no antimicrobial ingredients and a daily fluoride mouthrinse (Fluorigard, 0.05% NaF Colgate-Palmolive (UK) Ltd, Surrey, UK).

Visit 2: Six weeks later at the first adjustment appointment, the elastomers on the upper incisors and lower canines were aseptically removed, placed in separate containers with a pre-reduced transport medium and coded. These were taken to the laboratory within ten minutes. The adjustment to the appliance was carried out and non-fluoridated elastomers were placed on all the teeth to allow for a washout period of at least six weeks.

Visit 3: The appliance was adjusted and the fluoridated elastomers placed on the contra-lateral teeth to the first appointment. Therefore, if at appointment 2 the patient had the fluoridated module placed on the upper left incisors and lower right canine, at appointment 3 the fluoridated module was placed on the upper right incisors and lower left canine. Non-fluoridated elastomers were placed on the remaining teeth.

Visit 4: Six weeks later, the procedures carried out during appointment 2 were repeated.

Microbiology

On arrival in the laboratory each elastomeric sample that was collected on visit 2 and 4 was vortexed for 30 seconds, serially diluted and 100µl aliquots plated onto blood

agar (blood agar base number 2, Lab M, Bury Lancs supplemented with horse blood 5% v/v, Oxoid) and Mitis Salivarius (MS) agar (Difco, USA) supplemented with sucrose (20% w/v) and bacitracin (0.2 units/ml). Plates were incubated for up to 7 days at 37℃ either in CO_2 or anaerobically on pre-reduced plates under an atmosphere of 80% N_2, 10% H, 10%CO_2.

Cultures were assessed for total aerobic, total anaerobic and total Mitis Salivarius colony forming units (cfu). Wherever possible, cultures with colonies in the range 30-300 cfu were chosen for counting.

Representative colonies recovered on the Mitis Salivarius Bacitracin medium were Gram-stained to confirm that they were streptococci. Each streptococcal colony type was then speciated using the Rapid ID32 Strep system (bioMérieux, Basingstoke, UK). Although *Streptococcus sanguis* and *S.parasanguis* were recovered on the Mitis Salivarius Bacitracin agar plates in small numbers, growth was dominated by *S.mutans*. No *S.sobrinus* or other species of mutans streptococci were found with the identification system employed.

Statistics

Sample Size Calculation

A sample size calculation was carried out using data from two previous studies[4, 9]. This suggested that a sample size of 30 would be sufficient to detect a difference in the *Streptococcus mutans* count of 30 percent to a power of 0.85 with a significance level of 0.05.

Hypothesis Testing

The streptococcus count was expressed as a percentage of the total aerobic count. There were a number of occasions when no streptococci were recovered, therefore a logistic regression was carried out to assess whether there were any factors that affected the presence or absence of bacterial growth. The dependent variable was categorical yes or no indicating the presence or absence of bacterial growth. The covariates were subject, visit, fluoride or non-fluoride elastomeric, tooth type, dominant or non-dominant toothbrushing hand side, and number of days in the mouth. A mixed effects ANOVA was used to investigate the positive bacterial counts. The dependent variable was either the percentage streptococcal count or the anaerobic bacterial count, these were transformed to $\log_{10}$ values as they were found to be positively skewed. The random variable was the subject. The fixed factors included gender of patient, visit, fluoride or non-fluoride elastomeric, tooth type and dominant or non-dominant toothbrushing hand side. Covariates included age and the number of days the elastomer was in place.

Results

Thirty patients were recruited to the study. Three patients dropped out before any microbiology could be obtained. Several samples were lost due to failure of the fluoridated elastomers between appointments, debonding of brackets and irregularities in laboratory procedure. A total of 220 elastomers were collected from 27 patients. There were 18 females and 9 males. The average age was 14.2 years (sd 2.1, range 11.8 – 20.6). The elastomers were in the mouth for an average of 39.4 days (sd 9.0, range 28 – 67) for the first visit and 41.3 days (sd 9.1, range 28 – 63) for the second visit.

The results of the logistic regression are revealed in Table I. This shows that for the dependent variable, presence or absence of bacterial count, there were no significant independent variables for either the streptococcal count or the anaerobic growth. There were a higher proportion of negative streptococcal growths following the first visit (27%) than following the second visit (7%). The proportion of the total variation explained by both models was 94 percent, which suggests that the amount of variation not explained by the analysis was low and therefore these were good models to explain the presence or absence of streptococcal growth and anaerobic bacterial growth.

The results of the mixed effects analysis of variance are shown in Table II. There were significant results for the subject factor for percentage streptococcal and anaerobic growths ($p<0.001$). The only other significant factor for the percent streptococcal growth was whether the ligature was collected on the first or second visit.

Figure 1 shows two boxplots of the bacterial counts from the streptococcus growth media for ligatures collected on the first and second visits. This shows that the median and interquartile range of the streptococcal counts was greater for the ligatures collected on the second visit.

There was no significant effect of the fluoridated elastomeric ligatures on the bacterial growth either in terms of the percent streptococcal count ($p=0.288$) or the total anaerobic count ($p=0.230$). The bacterial counts were positively skewed and therefore the median and interquartile descriptors for the fluoridated and non-

fluoridated elastomers were calculated. These are revealed in Table III. Boxplots of these data are shown in Figure 2 and Figure 3.

Discussion

This study has shown that after a clinically relevant period of time in the mouth there were no significant differences in percent streptococcal or anaerobic bacterial counts in plaque obtained from fluoridated elastomers compared with conventional elastomers. It must be concluded that fluoridated elastomers are not effective at reducing streptococcal or anaerobic bacterial growth in plaque on a ligature and so probably also that surrounding an orthodontic bracket between adjustment visits.

The reason for this is unknown, but is most likely explained by the fact that fluoridated elastomers release high levels of fluoride initially, but this release rapidly drops to a point where it will not affect bacterial growth and metabolism[6]. The currently accepted view is that levels of fluoride that are bactericidal are far higher than are likely to be present in the mouth[10].

The short-term effects of fluoridated elastomers on the oral flora have been demonstrated previously. Wilson and Gregory[8] compared a group of patients wearing fluoridated elastomers with a group wearing conventional elastomers. They showed that the percent *Streptococcus mutans* count as a proportion of the total streptococcal count reduced significantly in unstimulated whole saliva samples after one week in individuals supplied with the fluoridated elastomers. However they found that the streptococcal count then rose to baseline levels in the second week.

Hallgren *et al*[9] looked at the effect on plaque of bonding the brackets with glass ionomer cement compared with conventional composite. Using a split mouth design on 12 individuals undergoing orthodontic treatment, they collected pooled plaque from test and control quadrants and found that the proportion of *Streptococcus mutans* was lower around brackets bonded with the glass ionomer cement. They also found that the concentration of lactic acid in plaque samples taken from glass ionomer cement was lower[11]. However, these findings might have been due to differences in the properties of the two materials other than their fluoride content[12].

Another reason for the apparent lack of effect of fluoridated elastomers on the plaque flora might be due to higher levels of bacteria found on the elastomeric material. Forsberg *et al*[13] found that most patients had a higher bacterial count on teeth ligated with conventional elastomers compared with teeth ligated with steel ligatures. In the present investigation it was noticed that clinically there was a marked deterioration in the physical properties of fluoridated elastomers in the mouth. They were considerably swollen compared with the conventional elastomers after six weeks and several were missing when the patient returned (Figure 4). This has been noted previously[14]. Wiltshire[15] found that the fluoridated elastomers doubled their weight after 1 month in the mouth, while the weight of the non-fluoride elastomers remained virtually unchanged. It could be that the plaque inhibiting effect of the fluoride is cancelled out by the deterioration in the physical properties of the elastomeric, which leads to a higher bacterial load in fluoridated elastomers.

In this investigation there was a higher proportion of samples with no streptococcal growth collected from the first visit compared with the second visit. This confirms the

work of Schiei *et al*[5] who found that there was a transient decrease in plaque and salivary *Streptococcus mutans* levels in the first few weeks of placing a fixed orthodontic appliance. They suggested that this was due to reduction in streptococcal reservoirs following the banding procedure. After three months the proportion of *Streptococcus mutans* had risen above pre-treatment levels.

Although fluoridated elastomers have not been shown to reduce the proportion of streptococci or number of anaerobic bacteria in plaque, they might help to reduce the prevalence and severity of demineralisation during orthodontics[16, 17] by raising the concentration of fluoride in the plaque adjacent to the bracket. This will help to restore a positive balance between remineralisation and demineralisation and would be a valuable area of further research.

Conclusion

Fluoridated elastomers are not effective at reducing streptococcal or anaerobic bacterial growth in local plaque surrounding an orthodontic bracket after a mean time of 40 days in the mouth.

Individual oral hygiene instruction for patients is likely to be more effective at reducing local plaque.

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Legends

Figures

Figure 1 - Boxplots showing median, interquartile and ranges of the percent streptococcal count for the samples collected at visit 2 and visit 4.

Figure 2 - Boxplots showing median, interquartile and ranges of the percent streptococcal count for the fluoridated and the non-fluoridated elastomers.

Figure 3 - Boxplots showing median, interquartile and ranges of the bacterial counts from the anaerobic growth media for the fluoridated and the non-fluoridated elastomers.

Figure 4 - Fluoridated elastomer on the upper right lateral incisor after six weeks in the mouth demonstrating the swollen appearance compared with the conventional elastomers on the other teeth. The fluoridated elastomer on the upper right central has been lost.

Tables

Table 1 - Results of the logistic regression showing the p values for the independent variables, where the dependent variable was the presence or absence of bacterial growth on the anaerobic or Streptococcus growth media.

Table II - Results of the mixed effects analysis of variance showing the p values for the independent variables, where the dependent variable is the percent streptococcal or anaerobic bacterial counts.

Table III - Descriptive data for the bacterial counts from the percent streptococcal and anaerobic bacterial counts for the fluoride and non-fluoride elastomers.

Figures

Figure 1

Boxplots showing median, interquartile and ranges of the percent streptococcal count for the samples collected at visit 2 and visit 4.

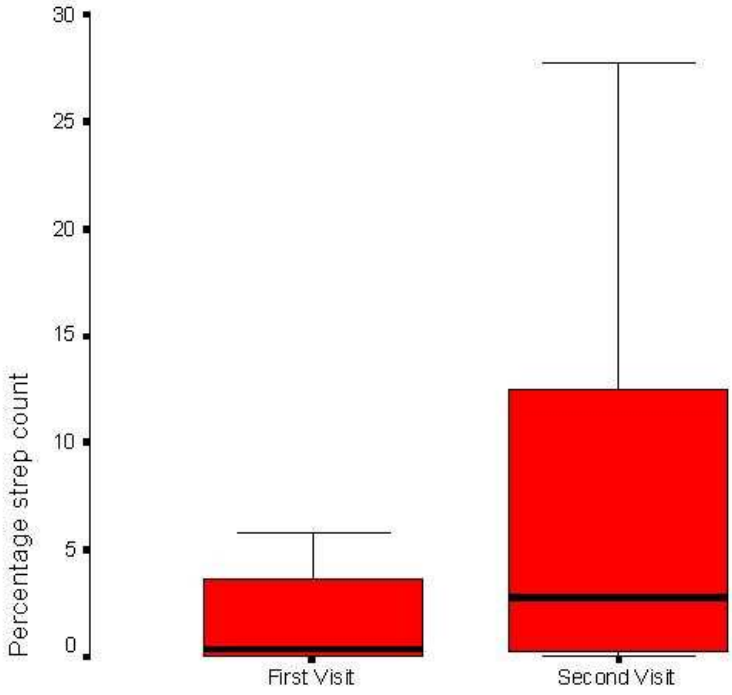


Figure 2

Boxplots showing median, interquartile and ranges of the percent streptococcal count for the fluoridated and the non-fluoridated elastomers.

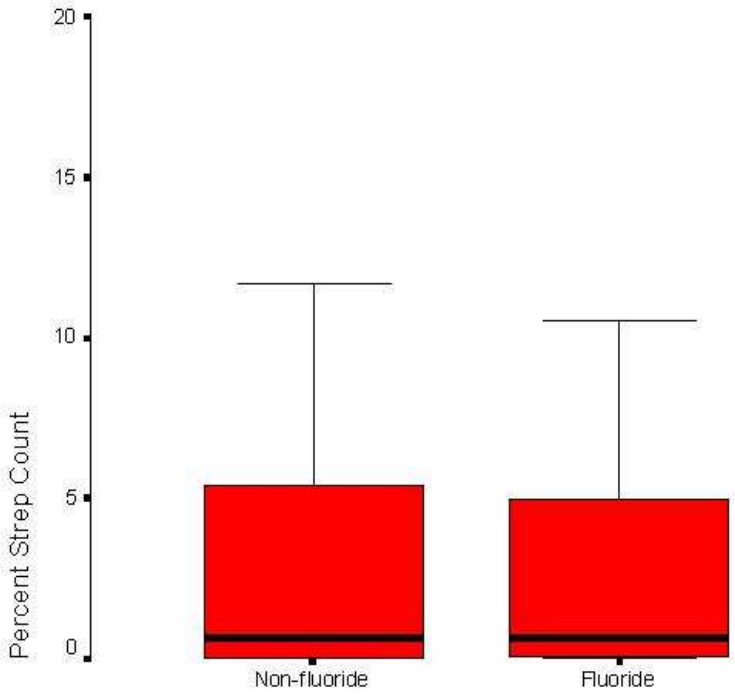


Figure 3

Boxplots showing median, interquartile and ranges of the bacterial counts from the anaerobic growth media for the fluoridated and the non-fluoridated elastomers.

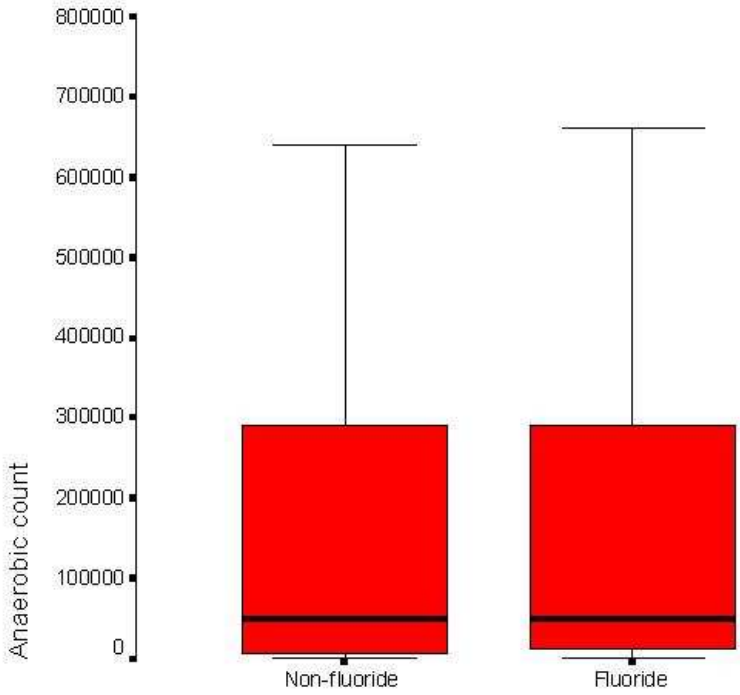
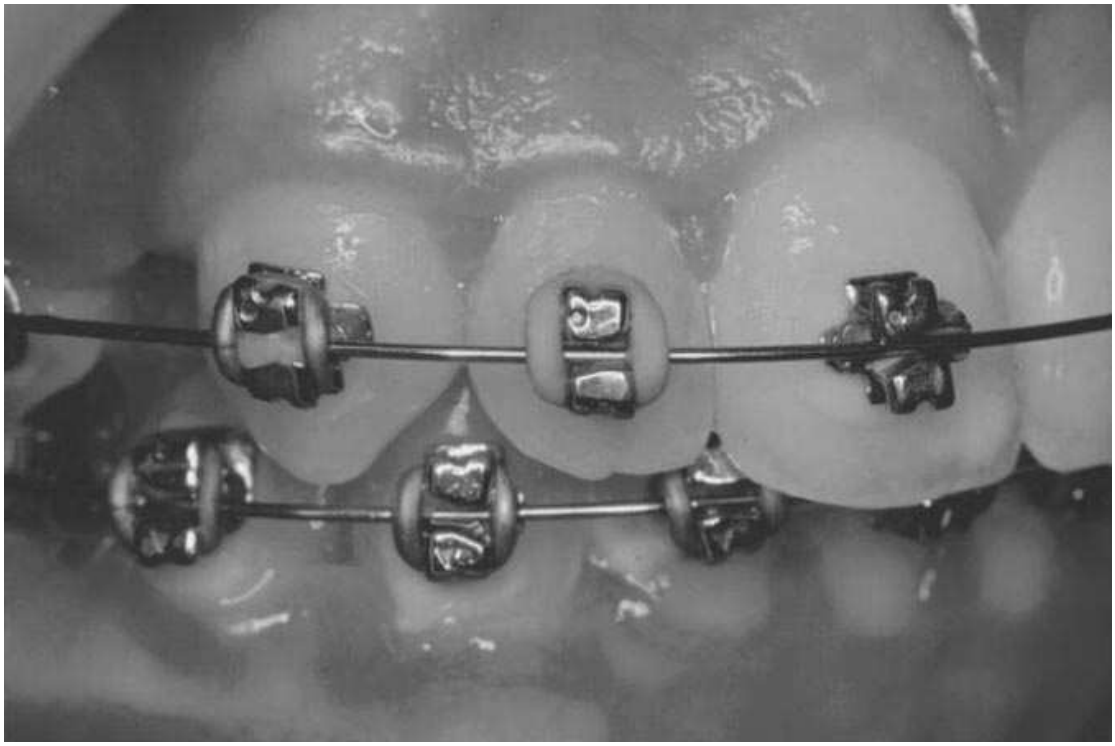


Figure 4

Fluoridated elastomer on the upper right lateral incisor after six weeks in the mouth demonstrating the swollen appearance compared with the conventional elastomers on the other teeth. The fluoridated elastomer on the upper right central has been lost.



Tables

Table I

Results of the logistic regression showing the p values for the independent variables, where the dependent variable was the presence or absence of bacterial growth on the anaerobic or Streptococcus growth media.

Independent Variable	Percent Streptococcal Count	Anaerobic Count
Subject	1.000	1.000
Fluoride or Non-fluoride elastomer	0.886	0.130
Tooth	0.961	0.573
Dominant or non-dominant hand	0.943	0.969
Visit 2 or 4	0.787	0.866
Number of days in mouth	0.105	0.880

Table II

Results of the mixed effects analysis of variance showing the p values for the independent variables, where the dependent variable is the percent streptococcal or anaerobic bacterial counts.

Independent Variable	Percent Streptococcal Count	Anaerobic Count
Fluoride	0.288	0.230
Tooth	0.394	0.346
Dominant	0.884	0.992
Visit	<0.001	0.301
Subject	<0.001	<0.001
Days	0.894	0.418
Age	0.283	0.307
Gender	0.530	0.937

Table III

Descriptive data for the bacterial counts from the percent streptococcal and anaerobic bacterial counts for the fluoride and non-fluoride elastomers.

	Percent Streptococcal Count		Anaerobic Count	
	Fluoride	**Non-fluoride**	**Fluoride**	**Non-fluoride**
Nos	104	110	111	111
Median	0.62%	0.66%	50,000	50,000
1st Quartile	0.03%	0%	12,000	5,000
3rd Quartile	4.97%	5.48%	290,000	300,000
Min	0%	0%	0	0
Max	91.7%	87.5%	8,300,000	77,000,000