

The effect of orthodontic treatment on salivary flow, pH, buffer capacity, and levels of mutans streptococci and lactobacilli

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Fixed orthodontic treatment has been shown to cause an increased incidence of enamel demineralisation. The purpose of this study was to investigate the changes in stimulated salivary flow rate, pH, buffer capacity, and the levels of mutans streptococci (MS) and lactobacilli in patients undergoing therapy with fixed orthodontic appliances. Saliva samples, plaque index scores and dietary histories were taken from 21 sequential patients before the start of treatment, and one month and three months after placement of brackets and bands. There was a statistically significant increase in stimulated salivary flow rate, pH, buffer capacity, plaque index scores, and in the levels of MS and lactobacilli after three months of active treatment. It is postulated that the balance between the cariogenic challenge posed by high levels of MS and lactobacilli, and the reparative effects of concurrent increases in salivary flow rate, pH and buffer capacity, determines the likelihood of mineral loss or gain over time. A failure to follow basic preventive measures may increase the risk for some patients of enamel decalcification during orthodontic treatment with fixed appliances. *Aust J Orthod* 1999; 15: 229–34

Introduction

Enamel decalcification is an undesirable but common complication of fixed appliance therapy. Several studies have noted an increase in the number of white spot lesions in patients undergoing therapy with fixed orthodontic appliances.^{1,2,3}

Decalcification occurs when the pH of the oral environment favours the diffusion of calcium and phosphate ions out of the enamel, following the production of acid by bacterial plaque.⁴ The inter-relationship between the various factors involved in the aetiology of mineral loss from enamel has traditionally been depicted as the co-existence of four factors: tooth, bacteria, substrate and time.⁵ However, the interactions between these factors are complex, and there is a multitude of factors that ultimately determine if demineralisation will occur.⁶

Mutans streptococci (MS) are strongly associated with the onset of dental caries; lactobacilli have been mainly associated with further development of the carious lesion in dentine.^{7,8,9} The placement of fixed orthodontic appliances results in a number of new retention sites for dental plaque, and several studies have shown increased levels of MS and lactobacilli in patients after fixed appliance placement.^{10,11,12,13,14}

Saliva is one of the most important modifying factors in the caries process. Salivary flow rate, salivary pH and buffer capacity contribute to the intra-oral acidogenic balance.⁵ During orthodontic treatment with fixed appliances, the highest incidence of decalcification occurs in the upper anterior teeth, a site with little exposure to saliva.¹⁵ This implies a relation between resistance to decalcification and salivary flow rate. The pH of saliva and its buffer capacity contribute to the ability of saliva to counter the acid produced locally in the oral cavity. A low pH intra-oral environment may favour colonisation by MS, which are aciduric;¹⁶ a higher salivary pH maintains a higher buffer capacity and is unfavourable to the growth of MS, which in turn leads to lower caries incidence.¹⁷ Salivary buffer capacity correlates directly with caries incidence.^{5,18}

While fixed orthodontic appliances appear to alter the ecology in the oral cavity (as seen in a transient increase in cariogenic bacteria), the duration of this increase has not been examined. Moreover, the impact of bracket placement on salivary factors, such as flow rate, pH and buffer capacity, has not been studied to any extent. A knowledge of such changes in patients undergoing orthodontic therapy with fixed appliances is important for understanding the process of decalcification, and for selecting appropriate preventive measures.¹⁹

Aims

The aim of the present investigation was to determine the effect of fixed orthodontic appliances on salivary flow rate, pH and buffer capacity, in conjunction with levels of MS and lactobacilli.

Subjects and methods

A total of twenty-one patients (4 males and 17 females, mean age 16 years, range 13-33 years), who were scheduled for treatment with fixed orthodontic appliances within the orthodontics clinic at the University of Queensland Dental School, participated in the study. Patients with any systemic disease, prescribed long-term medication, or in the mixed dentition phase were excluded. After the exclusion criteria were applied, patients were recruited into the study in a sequential fashion as they presented to the clinic for treatment. All patients or their guardians consented to participate after having received written information about the aims and design of the study. A power analysis conducted during the planning phase of the study indicated that a sample size of twenty patients would provide sufficient statistical power to discriminate changes in the categorical variables under study.

Before commencing fixed appliance treatment, patients were required to have all necessary general dentistry completed. Both the Edgewise and Begg lightwire orthodontic techniques were used. Intra-maxillary and/or intermaxillary elastics were used in some cases. The mean number of attachments placed in each patient was 16 (range 6-23). During active treatment, all patients were counselled regarding the importance of good oral hygiene and were instructed in toothbrushing techniques. All patients used fluoride-containing toothpaste; no supplementary fluoride was given.

Plaque scores and salivary samples were taken at the baseline assessment between two weeks and one week before banding, and subsequently one month and three months after placement of fixed orthodontic appliances. The time of day for sample collection for each individual was consistent during

the study. Eating and drinking were not permitted for one hour prior to sample collection. Subjects were instructed to brush only once in the morning on the day of sample collection.

Clinical recordings

Plaque index scores were recorded prior to the collection of saliva. The upper right first molar, lower right first premolar, lower right central incisor, upper left first premolar, upper left central incisor, and lower left first molar were scored for supra-gingival plaque on a 0-3 scale, according to the method of Löe.²⁰ In the event that any of these teeth were missing, the next tooth in the series was examined instead. Scores from the six index teeth were then added to give an overall score. The number of teeth with bands and brackets was recorded at each visit.

Dietary history

After each sample collection, patients were given a dietary history form, which was to be completed within seven days. Patients were required to record all food or beverages consumed for three successive days of their choice; however, at least one of these days had to be a Saturday or Sunday. The frequency of carbohydrate consumption per day was calculated and averaged for the three days.

Saliva sample collection and evaluation

Flow rate

Patients chewed bilaterally on a standardised piece of paraffin wax*. The saliva stimulated during the first 10 seconds was swallowed. The saliva stimulated thereafter was collected and the flow rate calculated (mL per minute) based on a collection time of five or ten minutes.

Salivary pH

The salivary pH was measured 30 to 60 minutes after its collection, using a digital pH-meter† that incorporated automatic temperature compensation. Preliminary studies had shown that salivary pH levels were stable after samples had been allowed to stand at room temperature for 30 minutes.

Buffer capacity

A pre-determined amount (0.1 mL) of 0.1 M acetic acid was added to 2.0 mL of saliva. The downward pH change, which is a measure of buffer capacity, was calculated by subtracting the original pH from the final pH.

Salivary levels of mutans streptococci

The Dentocult SM Strip mutans test‡ was used. This test has been shown to correlate highly with other methods, and to have good reliability.²¹ The

* Orion Diagnostica, Espoo, Finland.

† Piccolo ATC pH-meter, Hannah Instruments.

‡ Orion Diagnostica, Espoo, Finland.

test strip was inoculated with saliva and then incubated for 48 hours, according to the manufacturer's instructions. The number of MS per mL of saliva was estimated by comparing the test strip with the evaluation chart provided by the manufacturer. Counts were categorised as follows: score 0 = negative score; score 1 = less than 10^5 /mL; score 2 = 10^5 to 10^6 /mL; and score 3 = greater than 10^6 /mL.

Salivary levels of lactobacilli

The Dentocult LB dip-slide test[§] was used to estimate salivary lactobacilli levels. This method has good accuracy and reproducibility.²² The nutrient medium was inoculated according to the manufacturer's instructions. Following incubation at 35°C for four days, the number of lactobacilli per mL of saliva was estimated by comparing the colony density on the growth substrate with the template provided. The categories were as follows: score 0 = no colonies found; score 1 = 10^3 /mL; score 2 = 10^4 /mL; score 3 = 10^5 /mL; and score 4 = 10^6 /mL. Values greater than 10^4 /mL saliva were regarded as high (i.e. scores 3 and 4), and those less than 10^3 /mL saliva as low.

Data analysis

The non-parametric Wilcoxon matched-pairs test was used to determine the significance of differences between dependent samples. Possible associations between dependent and independent variables were analysed using the Spearman Rank order correlation test. The p values quoted are two-tailed.

Results

Levels of mutans streptococci and lactobacilli

There was a consistent trend for scores to increase after placement of fixed orthodontic appliances. Comparing scores across the group of patients by categories, the increase in levels of MS was significant ($p < 0.01$). As shown in Table I, none of the patients showed a decrease in scores, and only two patients retained a low score (score 1) status over the three months of the study. Other patients with a baseline score of 1 progressed to higher scores. Similarly, all but one of the eight patients who had a baseline score of 2 progressed to a score of 3 by three

Table I. Changes in scores for salivary Mutans streptococci at three months compared with baseline.

Score at baseline	Score at 3 months
1 (n= 4)	1 (n= 2)
	2 (n= 1)
	3 (n= 1)
2 (n= 8)	2 (n= 1)
	3 (n= 7)
3 (n= 9)	3 (n= 9)

The table shows the progression in scores over the period of the study. n = number of subjects

months. There was a consistent increase over time in the proportion of patients with very high levels of MS (score 3). At baseline, one month, and three months after banding, the number of patients with score 3 was 9, 13 and 17 out of 21, respectively.

Scores for lactobacilli increased rapidly after banding, and all 21 patients showed an increase (Table II). Across the group, the increase in scores at both one and three months was significant compared with baseline ($p < 0.001$). At baseline, only 2/21 patients had high scores (scores 3 or 4); after one and three months of banding, 14/21 and 20/21 patients respectively had high scores.

Salivary flow, salivary pH and buffer capacity

The median values for salivary flow rates, pH and buffer capacity are shown in Table III, together with the upper and lower quartiles. A comparison of the group data using non-parametric analysis demonstrated that there was a significant increase in the stimulated salivary flow rate after one month of banding ($p < 0.001$). Flow rates continued to rise after one month, with a further increase between the first and third month ($p < 0.05$). Salivary pH demonstrated a significant increase ($p < 0.001$) after three months. There was a significant rise in buffer capacity after one month ($p < 0.001$) of treatment, with no further changes that were significant between one and three months.

Table II. Distribution of scores for salivary lactobacilli.

	Lactobacillus Score				
	Score 0	Score 1	Score 2	Score 3*	Score 4*
0 months	1	12	6	0	2
1 month	0	3	4	2	12
3 months	0	0	1	5	15

The table shows the distribution of patient scores at each time period across each row (total n = 21). Data in each cell are the number of subjects with each score. The symbol * designates high scores.

[§] Orion Diagnostica, Espoo, Finland.

Table III. Summary data for salivary flow rate, pH, buffer capacity, plaque index and carbohydrate frequency.

	Time (month)	Median	Lower quartile	Upper quartile	P
Flow rate (mL/min)	Baseline	1.0	0.9	1.6	-
	1	1.7	1.4	2.2	<0.001
	3	2.1	1.2	2.7	<0.001
pH	Baseline	7.17	7.10	7.26	-
	1	7.18	7.10	7.30	ns
	3	7.34	7.20	7.47	<0.001
Buffer capacity	Baseline	0.77	0.62	0.96	-
	1	0.63	0.54	0.82	<0.05
	3	0.51	0.28	0.64	<0.01
Plaque index	Baseline	12	9	16	-
	1	19	15	22	<0.001
	3	20	16	21	<0.001
Carbohydrate frequency	Baseline	4.7	4.0	5.0	-
	1	4.3	3.3	4.9	ns
	3	4.7	3.5	5.0	ns

The non-parametric Wilcoxon matched-pairs test was used for statistical analysis. P values are for comparison of the 1 or 3 month intervals with baseline. ns = not significant.

Plaque levels and carbohydrate frequency

There was a significant increase in plaque scores after placement of fixed orthodontic appliances ($p < 0.001$, Table III). In contrast, the frequency of carbohydrate intake remained constant throughout the period of study for each patient (Table III).

Correlation between variables

Non-linear correlation analyses between the number of orthodontic attachments (independent variable) (Table V), and salivary flow rate, pH, buffer capacity, levels of MS and lactobacilli, and plaque levels, did not reveal any significant associations. Nor were plaque scores shown to correlate strongly with levels of MS. Although a significant correlation was found between plaque scores and lactobacillus levels after the first month ($p < 0.05$), this was not demonstrated at the third month. As expected, there was a trend for the buffer capacity to increase together with the salivary flow rate. At the end of the first month,

fifteen patients (71 per cent) demonstrated an increase in both the salivary flow rate and the buffer capacity (Table IV).

Discussion

This study has provided several new and valuable insights into changes in the oral environment that occur after the placement of fixed orthodontic appliances. Major changes occurred in salivary parameters, which may alter susceptibility to dental caries. In addition to clearing dietary substrates from the mouth, saliva buffers acids produced by dental plaque, and mediates exchanges in calcium and phosphate at the tooth surface as part of the intra-oral mineralisation-demineralisation balance.^{5,23} An elevation in flow rate will increase clearance of substrates, as well as the buffer capacity and the anti-bacterial activities of saliva.^{24,25}

The results of the present study support a direct and prolonged stimulatory effect of treatment with fixed

Table IV. Number of patients with an increase, decrease, or no change in salivary flow rate and buffer capacity at one month compared with baseline.

		Salivary Flow Increase	No change	Decrease	Total
Buffer capacity	Increase	15	2	—	17
	No change	—	1	—	1
	Decrease	2	—	1	3
	Total	17	3	1	21

Table V. Salivary parameters for individual subjects, arranged according to the number of appliances.

Appliances	Subject (Sex, Age)	Salivary flow, pH, buffer capacity		
		0 months	1 month	3 months
6	F 12.0 yrs	2.7, 7.32, 0.64	3.0, 7.30, 0.58	3.3, 7.47, 0.46
8	F 14.2 yrs	0.2, 7.03, 2.17	0.2, 6.87, 1.86	0.6, 7.02, 1.15
10	F 14.2 yrs	1.6, 7.33, 1.10	2.5, 7.40, 0.56	2.6, 7.46, 0.51
10	M 12.9 yrs	0.7, 7.06, 0.74	1.4, 7.10, 0.63	1.4, 7.07, 0.75
11	F 33.6 yrs	1.0, 7.13, 0.38	1.8, 7.05, 0.20	2.8, 7.37, 0.21
11	F 13.8 yrs	0.7, 7.25, 0.91	0.9, 7.29, 0.62	1.2, 7.50, 0.51
14	M 12.9 yrs	1.3, 7.08, 0.77	1.8, 7.18, 0.61	1.2, 7.31, 0.61
14	F 15.6 yrs	1.3, 7.27, 0.81	2.4, 7.16, 0.74	2.8, 7.40, 0.22
16	F 13.2 yrs	1.9, 7.10, 0.82	2.3, 7.01, 0.57	2.7, 7.24, 0.45
16	F 11.2 yrs	1.0, 7.39, 1.17	1.6, 7.31, 1.10	2.0, 7.58, 0.67
16	F 15.0 yrs	1.0, 7.30, 1.35	1.6, 7.33, 0.85	2.1, 7.51, 0.75
16	F 14.8 yrs	1.0, 7.18, 0.76	2.0, 7.16, 1.03	2.3, 7.33, 0.83
16	F 13.5 yrs	1.0, 7.10, 0.45	1.4, 7.05, 0.32	1.2, 7.12, 0.45
16	M 13.7 yrs	0.9, 7.12, 0.70	1.7, 7.24, 0.74	2.3, 7.34, 0.17
19	F 15.2 yrs	2.2, 7.15, 0.07	1.8, 7.30, 1.00	2.0, 7.43, 0.11
20	M 16.6 yrs	1.6, 7.17, 0.70	1.6, 7.11, 0.41	2.2, 7.20, 0.55
20	F 14.5 yrs	0.3, 7.19, 1.00	0.3, 7.16, 0.76	0.4, 7.21, 0.43
20	F 15.3 yrs	1.2, 7.27, 1.28	1.4, 7.10, 0.79	1.4, 7.14, 0.28
23	F 13.7 yrs	1.9, 7.11, 0.80	3.9, 7.20, 0.33	3.1, 7.23, 0.36
23	F 26.2 yrs	2.8, 7.17, 0.24	3.4, 7.69, 0.97	4.2, 7.75, 0.79
24	F 17.6 yrs	1.0, 7.18, 0.62	1.3, 7.49, 0.54	1.6, 7.67, 0.55

Data sets for salivary parameters contain 3 variables: salivary flow (in mL/min), pH, and buffer capacity (pH fall after addition of standardised amount of acid).

orthodontic appliances on salivary flow. While the salivary composition varies with the time of day and the individual's state of mind,²⁶ the conditions under which saliva was collected in this study were standardised as much as possible, so that these factors alone cannot be responsible for the changes seen.

With regard to salivary buffer capacity: after three months of treatment, there was a rise in salivary buffer capacity of between 40 to 80 per cent in 57 per cent of the patients. In keeping with previous studies of salivary biology,^{27,28} there was a tendency for buffer capacity to increase with salivary flow rate (Table IV), although some minor deviations from this pattern were seen.

In relation to cariogenic bacteria: the present study showed a rise in levels of MS in patients undergoing therapy with fixed orthodontic appliances. The increase was rapid, with the majority of patients (81 per cent) progressing to the highest possible score of three at the end of three months. The magnitude of the increase seen in the present study is in agreement with previous reports;^{13,12,29} however, the duration of the increase (three months and possibly beyond) is a novel observation. Whether the rate or magnitude of the increase relates to the number of brackets placed remains unclear. In the present study, patients with fewer attachments did not show a lesser rise in levels of MS. The minimum number

of attachments in the present study was six, and it would appear that this is sufficient to cause a change in the oral environment. Support for the concept of a threshold effect can be found in the study by Scheie *et al.*,¹³ in which a rise in MS occurred in all patients except those who had only two to four attachments. This point should be examined further.

In relation to salivary lactobacilli: the results of the present study demonstrate a substantial increase after only one month of active treatment. After three months of treatment, all patients had higher scores, and there was an 86 per cent increase in the number of patients with high levels, compared with the baseline assessment. Although lactobacilli do not play a significant role in the initiation of caries, their presence in large numbers indicates that the necessary conditions for producing dental caries do exist.³⁰

In the absence of significant dietary changes, the increase in MS and lactobacilli following placement of orthodontic attachments can be explained by the nature of their irregular surfaces, which promote plaque retention and the growth of these aciduric, acidogenic bacteria, which prefer hard surfaces on which to grow. The kinetics of the microbial changes in favour of caries-promoting bacteria within one month is consistent with reports of measurable demineralisation around fixed orthodontic appliances after only one month.³¹ Nevertheless, it

should be borne in mind that the relation between the amount of plaque and the severity of demineralisation is not straightforward,³² as the type of plaque plays a major role in determining pathogenicity.

In a recent report,¹⁹ several preventive strategies relating to changes in the oral environment in orthodontic patients were highlighted. While measurements of stimulated salivary flow rate, or other salivary parameters such as pH and buffer capacity, can be valuable as part of an overall clinical assessment of caries susceptibility,³³ these tests are regarded as less informative for caries prediction than microbial monitoring for MS.¹² Such monitoring may be of value to assess the impact of dietary control measures, and the effectiveness of anti-microbial therapies such as chlorhexidine.

In summary: the present study indicates that both favourable (salivary) and unfavourable (microbial) changes are elicited by the placement of fixed orthodontic appliances. When the balance between these competing influences on caries risk status¹⁹ is taken into account, the conflicting data in the orthodontic literature regarding the incidence of decalcification during treatment with fixed orthodontic appliances (ranging from two per cent to 96 per cent⁴) may be explained by the differing contributions of salivary and microbial factors in individual patients. Further studies are required to determine whether the trends seen in the present study carry through the duration of fixed appliance treatment, or reverse upon removal of the appliances. If orthodontic patients at high risk of decalcification can be identified at the commencement of treatment, preventive measures such as chlorhexidine gel therapy can then be implemented with maximal effect.

Acknowledgements

The authors thank Ivoclar (Melbourne, Australia) and Orion Diagnostica (Espoo, Finland) for supplying the Dentocult SM and Dentocult LB test kits.

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