# The effect of space maintainers on salivary pH, flow rate, and the oral microflora

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*Background:* Space maintainers are used to preserve created space caused by the premature loss of primary teeth but they may also upset the oral environment and play a role in caries formation. The current research aimed to assess the impact of removable and fixed space maintainers on salivary pH, flow rate, and the oral microflora.

Methods: Thirty-eight patients aged between 4 and 10 years, each of whom required a fixed (n = 19) or removable space maintainer (n = 19), were enrolled in this research. The salivary pH, saliva flow rate, salivary Streptococcus mutans, and Lactobacillus counts were measured immediately prior to the placement of the space maintainers (baseline-TO) and during the follow-up period, at the 1st (T1), 3rd (T3), and 6th (T6) month. The Wilcoxon, Mann–Whitney U test and Friedman tests were applied for statistical analyses.

*Results: Streptococcus mutans* and *Lactobacillus* counts were significantly higher at the 6th month time period in comparison with the baseline scores for both groups (P < 0.001). The salivary pH and flow rates did not change significantly at any measurement period (P > 0.05).

*Conclusions:* Space maintainers can favour caries formation by changing the oral microflora. It is advisable to warn patients and their parents of the risks and provide motivation to perform meticulous oral hygiene. (Aust Orthod J 2021; 37: 259 - 264. DOI: 10.21307/aoj-2021.028)

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### Introduction

Because a common aetiological factor for the development of a malocclusion is the premature loss of primary teeth and arch length contraction,<sup>1</sup> space maintainers are used to preserve arch length.<sup>2</sup> Space maintainer appliances may be fixed or removable depending on the requirements of the case. While fixed space maintainers are usually used to maintain arch integrity,<sup>2</sup> the loss of multiple primary molars due to caries or other reasons during the primary/ mixed dentition requires the use of removable space maintainers.<sup>3</sup>

Although the placement of a space maintainer is a preventive measure,<sup>2</sup> there are concerns regarding the impact of fixed and removable appliances on the oral

environment and the development of dental caries.<sup>4–6</sup> All space maintainers are plaque retentive since bands, brackets and wires increase the accumulation of debris and food on tooth surfaces,<sup>7–9</sup> reduce the cleansing effect of saliva<sup>7</sup> and make conventional oral hygiene more difficult. Therefore, space maintainers may facilitate dental disease.<sup>8,9</sup>

Dental caries represents a destructive multifactorial, infectious disease. Organic acids produced by oral microorganisms in dental plaque lower the pH to initiate demineralisation of teeth and initiate the carious process.<sup>10</sup> There are a considerable number of microorganisms reported to be responsible for acid production; however, two bacteria have been identified as dominant. *Streptococcus mutans (S. mutans)* is

known to be the primary cariogenic bacterium<sup>11,12</sup> and *Lactobacillus* is commonly associated with progression of the disease.<sup>11,12</sup> In addition, a reduced saliva flow rate influences caries formation and progression by affecting salivary pH and its buffering capacity.<sup>13,14</sup> Salivary flow provides a cleansing, buffering, and a remineralising action following initial decalcification.<sup>14,15</sup>

The aim of the present research was therefore to investigate the effect of fixed and removable space maintainers on *S. mutans* and *Lactobacillus* counts, the salivary pH and flow rates as contributory factors in the initiation and development of caries.

### Material and methods

The present research was conducted on 38 patients aged between 4 and 10 years, each of whom had a requirement for a fixed or removable space maintainer. The study was carried out in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Gazi University and the Department of Pediatric Dentistry at Ankara University. Patients with a medical history that could influence the oral microflora, including systemic disease, immunosuppression and the use of antibiotics during the preceding 15 days, were excluded from the research. The study divided the participants into two groups according to the inserted space maintainer and to achieve a power of 0.8 with an  $\alpha$  error of 0.05, the estimated number of participants was 18 for each group. To account for possible dropouts during follow-up, 40 patients were separated into the two groups: Group I (n = 20): fixed space maintainers; Group II (n = 20): removable appliances. To standardise the patient population, only a band-and-loop type of fixed space maintainer was inserted. All removable space maintainers incorporated an acrylic base and retention elements of either Adams' or C clasps. Due to a lack of co-operation and missed review visits, one patient from each group was not included in the study. Therefore, final participation was comprised of 19 patients in each group for a total of 38 patients (Group I: 9 boys and 10 girls, Group II: 8 girls and 11 boys).

Approval was received from the ethics committee of the University and informed consent was obtained from the children and the participant's parents. Oral hygiene education for the patients plus their parents was provided by a paediatric dentist [E.K.] during a treatment session 1 month before the placement of the appliances. For standardisation, the oral hygiene of all of the patients was monitored by the parents during the study period. The measurements presented below were performed immediately after the space maintainers were applied at baseline (T0) and at the 1st (T1), 3rd (T3), and 6th (T6) month period after commencement.

Salivary flow rate was estimated by stimulated samples provided by all children between 10 a.m. and 12 noon. The patients were instructed not to eat, drink nor brush their teeth at least 2 hr prior to saliva collection. Each child was asked to sit in a chair and rest for a few minutes following which, each was provided with paraffin wax for chewing. During the first 30 sec, the produced saliva was swallowed. Subsequently, the saliva was collected into pre-weighed, sterile, plastic containers for a period of 5 min.<sup>5</sup> The sterile plastic containers containing the collected saliva were weighed and the flow rate calculated in gms/min which was accepted as approximately equivalent to ml/min.<sup>16</sup> The pH was measured after saliva collection using a calibrated pH meter (Corning-450, Corning NY, USA).

The samples were kept on ice and immediately sent to the microbiology laboratory for isolation and quantitation of the microorganisms. Salivary tests and microbial analysis were performed on the same day.

# Microbial sampling, isolation, and cultivation of S. mutans and Lactobacillus

The saliva samples were vortexed in order to obtain a homogeneous suspension, and 10-fold serial dilutions of each sample were cultivated on Mitis Salivarius Agar (MA; Sigma) and Rogosa agar (RA; Sigma) as triplicates. Isolation controls from each sample were performed by Mitis Salivarius Agar (MA; Sigma) and Rogosa Agar (RA; Sigma) in parallel.

The saliva samples were used to assess the numbers of *S. mutans* and *Lactobacilli* isolates. The plates were incubated in an atmosphere of 5% CO<sub>2</sub> at 37°C for 48 hr. The colonies were morphologically examined by gram staining.<sup>17</sup> The culture suspensions of *S. mutans* isolates were produced on Todd-Hewitt Broth agar (THB; Sigma) and *Lactobacilli* on MRS Broth (DeMan-Rogosa-Sharpe; Sigma) in 5% CO<sub>2</sub> at 37°C for 48 hr.<sup>18</sup> The numbers of colony-forming units per ml (CFU/ml) of *S. mutans* were counted on Mitis Salivarius agar and *Lactobacilli* were counted on Rogosa agar, respectively.

#### **Statistics**

SPSS version 11.5 for Windows (SPSS Inc., Chicago, Illinois, USA) was utilised for statistical analysis. The dependent (Wilcoxon test) and independent sample (Mann–Whitney U test) and Friedman test were applied for investigating the relationships between microorganism counts, changes in the flow rate and salivary pH. The *P*-value levels set for the research were P < 0.05, P < 0.01, and P < 0.001.

### Results

The salivary pH, stimulated saliva flow rate, *S. mutans* and *Lactobacillus* counts in saliva measured at T0, T1, T3, T6 follow-up periods are shown in Table I for the space maintainer groups.

Salivary pH: in Group I (fixed space maintainer), the salivary pH value increased at T1 (7.25  $\pm$  0.28).

The salivary pH decreased at the T3 (7.23  $\pm$  0.31) and T6 (7.22  $\pm$  0.27) periods and subsequently returned to the T0 value. For Group 1, alterations in the flow rate did not significantly differ at any of the measurement periods (T0–T1, T0–T3, T0–T6) (*P* > 0.05).

In Group II (removable space maintainer), salivary pH decreased at the T1 (7.16  $\pm$  0.16) and T3 (7.11  $\pm$  0.26) periods. The salivary flow rate increased at the T6 (7.17  $\pm$  0.18) period and approached the T0 value.

Alterations in the salivary pH for Group II did not differ significantly at any of the measurement periods (T0-T1, T0-T3, T0-T6) (*P* > 0.05).

Flow rate: salivary flow rate was  $3.56 \pm 0.89$  and  $3.09 \pm 0.94$  mls/min for Group I and Group II, respectively, at the T0 period.

For Group I, salivary flow rate increased at T1 (3.74  $\pm$  0.79). The salivary flow rate decreased at the T3 (3.63  $\pm$  0.82) and T6 (3.57  $\pm$  0.81) periods and approached the T0 value. For Group 1, alterations in the flow rate did not differ significantly at any of the measurement periods (T0–T1, T0–T3, T0–T6) (*P* > 0.05).

The flow rate increased during the follow-up period in those patients who used removable space maintainers. For Group II, alterations in the flow rate did not differ significantly at any of the measurement periods (T0–T1, T0–T3, T0–T6) (P > 0.05).

## S. mutans and Lactobacillus counts in saliva

S. mutans counts in saliva were  $5.09 \pm 2.32 \times 10^5$  colonyforming units per ml (CFU/ml) and  $4.77 \pm 2.91 \times 10^5$ CFU/ml for Group I and Group II, respectively, at the T0 period. The level of S. mutans in saliva was found to increase during the follow-up period in patients who either used fixed or removable space maintainers (Table I). In Group I, the S. mutans count in saliva at T3 and T6 periods was significantly higher than S. mutans count at T0 and T1. In Group II, statistically significant differences were noted in S. mutans counts at all of the follow-up periods (P < 0.001) (Table I).

*Lactobacillus* counts in saliva were  $0.31 \pm 0.26 \times 10^5$  CFU/ml and  $0.43 \pm 0.597 \times 10^5$  CFU/ml for Group

	S. mutans (×10 <sup>5</sup> ) CFU/ml		Lactobacillus (×10⁵) CFU/ml	
	Group   ( <i>n</i> = 19)	Group II ( <i>n</i> = 19)	Group I (n = 19)	Group II ( <i>n</i> = 19)
TO Mean ± SD	5.09 ± 2.32	4.77 ± 2.91	0.31 ± 0.26	0.43 ± 0.59
T1 Mean ± SD	5.28 ± 2.15	5.05 ± 3.18	0.29 ± 0.22	0.49 ± 0.62
T3 Mean ± SD	78.19 ± 31.07	53.15 ± 32.97	1.68 ± 1.28	2.25 ± 3.07
T6 Mean ± SD	79.64 ± 27.42	54.52 ± 33.82	1.42 ± 1.05	2.45 ± 3.70
Friedman	42.67	48.87	41.03	48.98
Р	0.000***	0.000***	0.000***	0.000***
Difference	TO, T1–T3, T6	TO-T1, T3, T6	TO, T1–T3, T6	TO-T1, T3, T6
		T1-T3, T6		T1-T3, T6
		T3-T6		Т3-Т6

Table I. Comparison of the changes in S. mutans and Lactobacillus counts for fixed and removable appliances groups throughout the study.

Follow-up periods: baseline – T0, 1st – T1, 3rd – T3, and 6th – T6 months. SD: standard deviation. \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

I and Group II, respectively, at the T0 period. In Group I, the *Lactobacillus* count in saliva at T3 and T6 periods was significantly higher than the *Lactobacillus* count at T0 and T1. In Group II, statistically significant differences were found in the *Lactobacillus* counts at all of the follow-up periods (P < 0.001) (Table I).

### Discussion

Dental caries is a dynamic process involving complex interactions between cariogenic bacteria and fermentable carbohydrate over the course of time.<sup>19</sup> Salivary characteristics including pH, flow rate and buffering capacity are significant host risk factors<sup>15,19</sup> and *S. mutans* and *Lactobacillus* are regarded to be the major pathogens.<sup>8</sup> The current study aimed to assess possible alterations in these factors following the insertion of space maintainers.

The present study was conducted on 38 paediatric patients aged between 4 and 10 years. While most studies have examined the impact of orthodontic appliances on periodontal health and plaque accumulation in patients aged between 12 and 27 years,<sup>20,21</sup> space maintainers are usually placed during the developing dentition. Ineffective oral hygiene has been reported in children under the age of 10 years which has been attributed to poor dexterity and a lack of motivation.<sup>22</sup> This is consistent with behaviour, personality, and self-image development during adolescence.23 Therefore, because the oral hygiene status of younger patients was expected to be poor, for standardisation, the oral hygiene practices for all of the subjects were performed by their parents throughout the study period.

Biological elements/factors in saliva, of which salivary flow rate and pH are regarded as important, protect the teeth from caries development and allow remineralisation.<sup>15,24</sup> Previous studies have reported that the average level of saliva flow and pH is elevated in caries-free children in comparison with caries active children; however, the difference is considered to be statistically insignificant.<sup>15,24</sup>

There are conflicting reports regarding salivary flow and pH changes in patients undergoing fixed orthodontic treatment, either asserting increases<sup>25,26</sup> or no change.<sup>27</sup> Lara-Carrillo et al. reported that the stimulated salivary flow rate and pH significantly increased 1 month after the commencement of orthodontic treatment. If an oral foreign body is present, salivary flow increases and modifies salivary composition to increase the pH. These protective effects provide an oral environment conducive for the colonisation of pathogenic microorganisms.<sup>28</sup> In the present study, although the salivary flow rate and pH increased at T1 (the 1st month) in the fixed space maintainer group, no significant differences were noted in the salivary flow rate and pH at the end of the follow-up period when compared to the baseline assessment. Similarly, Bonetti et al. found no statistically significant difference after a 1-year followup for salivary flow rate and pH parameters following fixed orthodontic treatment. It was considered that salivary parameters could be changed during the early period of treatment.<sup>27</sup> However, a comparison of the results with studies that evaluated changes during fixed orthodontic treatment is inappropriate because of the differences in the number of intraoral attachments. It is necessary to conduct additional studies to investigate the long-term impact of space maintainers on saliva parameters.

The present study also examined whether surface colonisation by *S. mutans* and *Lactobacillus* was influenced by the placement of space maintainers. According to the results, *S. mutans* and *Lactobacillus* counts increased significantly throughout the study period for Group II in which *S. mutans* counts in saliva at T3 and T6 periods were significantly higher than *S. mutans* counts at T0 and T1.

An increase in plaque and biofilm formation predisposes to increased microorganism colonisation. Plaque accumulation and gingival irritation can occur in association with the use of bands, brackets, wires, and acrylic resins, so that the oral flora responds, especially if oral hygiene is poor.<sup>9,28,29</sup> Several studies have indicated that orthodontic treatment increases the risk of food debris and microorganism retention,<sup>21,28,29</sup> but few studies have recorded the relationship between microorganisms and space maintainers.<sup>30</sup>

Arıkan et al. investigated the impact of fixed and removable space maintainers on the plaque index scores of children, and a positive correlation was shown for both appliances between the baseline and the 3rd and 6th periods.<sup>9</sup> A further study provided evidence supporting the findings from a baseline to 9 months.<sup>30</sup>

Boyd and Baumrind determined plaque index scores during the use of brackets or bands on molars, and significantly higher plaque index scores were revealed in association with banded molars over the treatment periods.<sup>31</sup> Arıkan et al. observed that fixed space maintainers led to increased plaque accumulation whereas removable space maintainers did not but both space maintainer types increased the number of microorganisms, in deference to periodontal health.<sup>9</sup>

According to El-Patal et al., *S. mutans* levels increase significantly in patients with attached band-loop space maintainers during a 6-month follow-up period. However, no significant increase in the *Lactobacillus* count has been observed during the same period.<sup>6</sup> Kundu et al. showed that the use of fixed space maintainers and removable appliances caused an increase in bacterial colonisation (*S. mutans, Lactobacillus*) during an orthodontic therapy period involving the first 6 months.<sup>32</sup>

The present study has noted limitations. Orthodontic treatment can cause mouth irritation, pain, functional problems, and displeasure.<sup>33,34</sup> Patients tend to consume soft foods and drinks otherwise mastication induces discomfort.<sup>33,35</sup> Özdemir et al. reported that orthodontic treatment caused changes in diet especially in the first weeks of treatment. Even at the 12th week of treatment, dietary habits did not return to initial levels.<sup>35</sup> Llena and Forner showed that sweet snacks, bread and soft drink consumption showed a positive association with caries.<sup>36</sup>

No study examining the relationship between the use of space maintainers and dietary habits has been identified. That changes may occur in eating habits were not evaluated in the present study and remain a limitation. The use of space maintainers may lead children to consume soft food or unilaterally chew, which are associated responses to fixed appliance treatment. Therefore, without adequate oral hygiene, the risk of dental caries and the levels of cariogenic bacteria may increase. Further studies to evaluate food consumption during space maintainer treatment is recommended.

### Conclusions

Space maintainer appliances increase pathogenic microorganism counts, causing an increased risk of dental caries. Patients and their parents should be advised regarding possible risk factors and should be instructed and motivated in correct oral hygiene practices during and beyond the treatment period.

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### **Conflict of Interest**

The authors declare that there is no conflict of interest.

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