Original Paper

Effects of Fixed and Removable Space Maintainers on Plaque Accumulation, Periodontal Health, Candidal and *Enterococcus Faecalis* Carriage

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Abstract

Objective: To evaluate the effects of space maintainers on plaque accumulation, periodontal health and oral microflora. *Subjects and Methods:* The study participants comprised 38 patients aged 4–10 years requiring either fixed or removable space maintainers. Plaque index, gingival index, bleeding on probing index, candidal colonization and *Enterococcus faecalis* were recorded just before the application of space maintainers (T0) and during treatment at the 1st (T1), 3rd (T2) and 6th (T3) month. *Results:* The gingival and bleeding on probing index scores increased significantly (gingival index from 0.20 ± 0254 to 0.54 ± 0417 and bleeding on probing index from 7.18 ± 9.946 to 18.07 ± 14.074) in the regions with fixed space maintainers at T3 ($p < 0.01$). The mean *Candida* counts also increased (for removable appliances from 1.90 ± 3.638 to 1.98 ± 3.318, $p < 0.05$, and for fixed appliances from 4.25 ± 4.587 to 4.52 ± 4.431, $p < 0.001$). The salivary *E. faecalis* counts at T3 also increased significantly with the use of fixed and removable appliances (for removable appliances from 5.93 ± 2.65 to 85.53 ± 34.1 and for fixed appliances from 4.95 ± 2.94 to 123.59 ± 29.51, $p < 0.001$). A positive correlation was found between the plaque ($r = 0.67$), gingival ($r = 0.76$) and bleeding on probing index scores ($r = 0.76$) and the candidal colonization for the fixed space maintainers ($p < 0.01$, $p < 0.001$). *Conclusions:* In this study, both fixed and removable space maintainers led to an increase in the number of microorganisms in the oral cavity as well as to increases in the periodontal index scores. Patients should be informed that space maintainers may serve as a source of infection and that special attention must be given to their oral hygiene.

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Introduction

Space maintainers are typically used in pediatric dentistry to preserve the spaces left by primary teeth requiring extraction prior to their exfoliation time [1, 2]. Space maintainers may be fixed or removable, and they are generally used in cases of an early extraction of 1st and 2nd primary molars [3]. Although it is well known that the maintenance of these spaces prevents later complications such as crowding, ectopic eruption, impaction of successor teeth and malocclusion [4–6], the use of space main-

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tainers has also been shown to result in an increased plaque accumulation, which can lead to dental caries and periodontal disease [7, 8].

Dental plaque is a complex substance comprised of bacteria in a biofilm formation that provides nutrients to the bacteria through a primitive vessel system [9]. The presence of bacterial plaque due to poor oral hygiene is the primary cause of gingival inflammation and periodontitis in children as well as adults [10, 11]. The retention of plaque and the development of gingivitis are dramatically affected by local factors [8]. Orthodontic bands and brackets are reported to influence plaque growth and maturation [12], and orthodontic appliances are reported to promote plaque accumulation and cause gingivitis [7, 13]. Periodontal pathological changes due to plaque accumulation range from reversible changes to significant attachment loss during orthodontic treatment [7, 12–14].

Recently, poor oral hygiene has been reported to promote oral carriage of the antimicrobial-resistant microorganism *Enterococcus faecalis* in individuals with fixed orthodontic appliances [15]. Not only is *E. faecalis* reported to play a role in endodontic treatment failure [16], but there is also a well-known correlation between periodontal disease and *E. faecalis,* which can cause systemic infections as well [17].

Numerous studies have reported on the increased oral colonization by candidal species in patients using dentures and orthodontic appliances [6, 18, 19]; however, there are no published studies relating to candidal colonization in conjunction with the use of fixed and removable space maintainers. Moreover, despite the similarities between fixed and removable space maintainers and orthodontic appliances, studies examining the effects of orthodontic appliances on periodontal health and the presence of oral microorganisms have mainly been conducted with adolescents [6, 19–21]. However, the age profile of the patients using space maintainers tends to be much younger, and the effects of plaque accumulation due to possible poor compliance to oral hygiene instructions among younger patients may be much worse. In spite of the importance of this issue, there is only one published study [8] investigating the effects of space maintainers on plaque accumulation and periodontal health, and there is no report on mucosal *Candida* and salivary *E. faecalis* levels in young patients using fixed or removable space maintainers. Thus, the present study was aimed at investigating the effects of fixed and removable space maintainers on plaque formation, periodontal health and on mucosal candidal and salivary *E. faecalis* counts in young patients.

Material and Methods

This study was conducted at two centers, at the Department of Pediatric Dentistry, Ankara University, and at the Department of Pharmaceutic Microbiology, Faculty of Pharmacy, Gazi University, Ankara, Turkey, on patients aged 4–10 years for whom fixed or removable space maintainers were indicated. According to the power analysis that was performed before the study, the estimated number of participants was 18 for each group, with an α error of 0.05 and a power of 0.8. Considering a worst-case scenario with possible losses during the follow-up period for each group, 20 patients who had an indication of a fixed (group I) or of a removable space maintainer (group II) on 1 jaw were randomly selected. Patients were excluded if they had a medical history that might affect candidal carriage such as systemic disease, immunosuppression and antibiotics use in the previous 15 days. To standardize the patient population, only band-and-loop type space maintainers were included in the fixed space maintainer group. All removable space maintainers were made of an acrylic base and retention elements (vestibul arch, Adam's and C clasps). One patient in each group was excluded from the study due to lack of cooperation with respect to the follow-up visits; therefore, the study was completed with 19 patients in each group and 38 patients in total (group I: 9 boys and 10 girls, group II: 8 girls and 11 boys).

The study was approved by the university's ethics committee, and written informed consent was received from all participants and their parents. A pediatric dentist (E.K.) provided oral hygiene education to the parents 1 month prior to the insertion of the space maintainers. The education consisted of 2 slideshows (1 for the patient and the other for the parents) and of a description of the mechanical cleaning of the teeth of models. The slideshows included information about oral hygiene, toothbrushing, dental flosses and the role of nutrition on oral health. For standardization, the oral hygiene of all the patients was provided by their parents throughout the study. The following periodontal measurements were done on all teeth and were performed just before (baseline; T0) the insertion of space maintainers and at the 1st (T1), 3rd (T2) and 6th month (T3) of treatment. (a) Dental plaque index: dental plaque was measured using a sterile periodontal probe according to Silness and Loe [22]. Separate scores were obtained for each tooth. (b) Gingival index: the gingival index measurements were obtained using a sterile periodontal probe [21]. (c) Bleeding on probing index: bleeding on probing was measured using a sterile periodontal probe. Two pediatric dentists (V.A. and E.K.) did the examinations for the dental plaque index, gingival index and bleeding on probing index. Before the study, 2 training sessions were done for calibration on 10 patients for each index. The κ scores for each variable ranged between 0.9 and 1, indicating almost perfect agreement in the 2nd session. (d) Oral candidal carriage: *Candida* samples were taken from 6 intraoral mucosal sites (anterior palate, posterior palate, anterior tongue, posterior tongue, left cheek, right cheek) using the imprint culture method [7]. In brief, sterile foam pads soaked in Sabouraud's broth were applied to each mucosal surface and then placed with the contact side down on Sabouraud's agar (Oxoid). The agar plates were then incubated aerobically at 37°C for 48 h, the foam pads were removed, and the plates were reincubated for an additional 72 h. The candidal colonies were counted separately for each site by visual examination and expressed as colony-forming units (CFU)/mm^2 [23]. (e) *E. faecalis* in saliva: after rinsing with 10 ml of phosphate-buffered saline (0.1 M/pH: 7.2), sam-

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Table 1. Comparison of the changes in plaque, gingival and bleeding on probing index scores for fixed and removable appliances groups throughout the study

	Plaque index			Gingival index			Bleeding on probing		
	with s.m. (n = 19)	without s.m. (n = 19)	p	with s.m. (n = 19)	without s.m. (n = 19)	p	with s.m. (n = 19)	without s.m. (n = 19)	p
Group II									
T0–T1	0.13±0.14	0.11±0.17	0.56	0.25±0.34	0.30±0.42	0.76	9.16±12.03	9.11±14.33	0.98
T0–T2	0.15±0.17	0.13±0.10	0.61	0.28±0.37	0.21±0.39	0.23	8.90±13.29	4.00±16.05	0.07
T0–T3	0.22±0.46	0.10±0.09	0.27	0.10±0.26	0.04±0.23	0.24	4.68±9.74	−0.34±11.82	0.05
Group I									
T0–T1	0.11±0.13	0.05±0.07	0.04*	0.20±0.25	0.07±0.14	0.04*	7.18±9.94	2.53±6.26	0.09
T0–T2	0.20±0.24	0.07±0.08	0.01*	0.38±0.43	0.17±0.15	0.02*	15.41±21.23	5.95±6.65	0.03*
T0–T3	0.21±0.35	0.13±0.37	0.34	0.54±0.41	0.18±0.27	0.0***	18.07±14.07	5.07±8.23	0.001**

s.m. = Space maintainer. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

ples were collected in sterile containers and concentrated by centrifugation at 17,000 *g* for 10 min according to Samaranayake et al. [24]. *E. faecalis* was isolated on MacConkey's agar (Oxoid) and then cultured in brain heart infusion agar (Merck) supplemented with 5% blood. Incubation was performed in a microaerofilic atmosphere (Anaerocult C, Merck) at 37°C for 2 days. The colonies were counted macroscopically based on characteristic Gram stain morphology, and the number of CFUs was calculated by dividing the number of CFUs counted by the dilution factor and recorded as CFU/ml of the original saliva sample [24].

The statistical analysis was performed using SPSS version 11.5 for Windows (SPSS Inc., Chicago, Ill., USA). Dependent and independent t tests and Pearson's correlation analysis were used to examine any correlations between the candidal counts and the plaque, gingival and bleeding on probing index. $p > 0.05$ and the parameters were fit to a normal distribution according to the Kolmogorov-Smirnov test. The level of the p value set for the study was * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

The plaque, gingival and bleeding on probing index scores were analyzed separately by jaw (maxillary, mandibular) in patients with removable space maintainers and by jaw and side (maxillary right, maxillary left, mandibular right, mandibular left) in patients with fixed space maintainers, and these regional scores were compared with those of regions where no space maintainers or retention elements were present.

Results

Plaque Index

In group I, the regions with space maintainers showed significantly greater changes in the plaque index than the regions without space maintainers from T0 to T1 (with space maintainers 0.11 ± 0.13, without space maintainers 0.05 ± 0.07; $p < 0.05$) and from T0 to T2 (with space maintainers 0.20 ± 0.24, without space maintainers 0.07 ± 0.08;

$p < 0.05$); however, the changes in the plaque index from T0 to T3 did not differ significantly between the 2 regions. In group II, the changes in the plaque index did not differ significantly between the regions with and without space maintainers at any of the measurement periods ($p > 0.05$) (table 1).

Gingival Index

In group I, the regions with space maintainers showed significantly greater changes in the gingival index than the regions without space maintainers from T0 to T1, T0 to T2 and T0 to T3 ($p < 0.05$). In group II, the changes in the gingival index did not differ significantly between the regions with and without space maintainers at any of the measurement periods ($p > 0.05$) (table 1).

Bleeding on Probing

In group I, the changes in the bleeding on probing scores did not differ significantly between the regions with space maintainers and those without space maintainers from T0 to T1; however, the regions with space maintainers showed significantly greater changes in the bleeding on probing scores than the regions without space maintainers from T0 to T2 and from T0 to T3 ($p < 0.05$). In group II, the changes in the bleeding on probing scores did not differ significantly between the regions with and without space maintainers at any of the measurement periods ($p > 0.05$) (table 1). Salivary *E. faecalis* counts increased over T0 were observed for patients using removable and fixed space maintainers (removable space maintainers, T0: 5.93 ± 2.654, T1: 6.69 ± 4,191, T2: 79.51 ± 24.004, T3: 85.53 ± 34.099; fixed space maintainers, T0:

Table 2. Changes in the total and mean candidal counts of fixed and removable appliance groups at T0 and during follow-up

	Mean *Candida*		Total *Candida*	
	group II	group I	group II	group I
T0	0.44±1.51	0.70±2.28	1.22±4.49	3.16±12.82
T1	0.94±2.87	0.57±1.44	2.56±8.50	1.84±5.37
p	0.16	0.68	0.19	0.47
T2	1.90±3.63	4.25±4.58	7.61±15.63	11.79±13.94
p	0.12	0.003**	0.11	0.01*
T3	1.98±3.31	4.52±4.43	7.06±14.12	17.32±21.19
p	0.04*	0.00***	0.07	0.00***

* p < 0.05; ** p < 0.01; *** p < 0.001.

Table 3. Number of patients with candidal colonization at T0 and during follow-up, by site

	Buccal		Palatal		Lingual	
	right	left	posterior	anterior	posterior	anterior
Group II						
T0	0	1	0	0	2	2
T1	0	1	1	0	3	2
T2	9	6	2	4	5	4
T3	6	5	2	1	5	2
Group I						
T0	1	2	1	1	1	1
T1	3	4	0	0	2	2
T2	17	16	2	1	4	4
T3	16	17	2	2	12	10

Table 4. Correlations between the plaque, gingival and bleeding on probing index scores and the candidal colonization at T0 and during follow-up

		Plaque index	Gingival index	Bleeding on probing
T0 *Candida*				
Group II	r	0.76	0.93	0.953
	p	0.00***	0.00***	0.00***
Group I	r	0.03	0.36	0.38
	p	0.89	0.12	0.10
T1 *Candida*				
Group II	r	0.51	0.55	0.63
	p	0.03*	0.01*	0.00***
Group I	r	−0.19	0.09	0.24
	p	0.41	0.71	0.31
T2 *Candida*				
Group II	r	0.49	0.32	0.54
	p	0.03*	0.18	0.02*
Group I	r	0.60	0.52	0.48
	p	0.00***	0.02*	0.03*
T3 *Candida*				
Group II	r	0.09	0.11	0.19
	p	0.69	0.65	0.43
Group I	r	0.67	0.76	0.76
	p	0.00***	0.00***	0.00***

* p < 0. 05; ** p < 0. 01; *** p < 0.001.

creased significantly over T0 at T2 and at T3 in both groups. The candidal counts also increased at the anterior lingual mucosa at T3 in group I. The number of sites with candidal colonization increased in both groups, with no significant differences between the groups at T0 or at T2. However, at T3, group I showed a statistically higher number of *Candida*-positive sites when compared to group II.

No statistically significant differences were found between the groups in the number of patients with candidal colonization at any of the buccal and lingual sites at T0 or T1 (table 3). However, at T2 and T3, the number of patients with fixed space maintainers who had candidal colonization in both left and right buccal mucosa sites was significantly higher in comparison to the number of patients with removable space maintainers (p < 0.05), and at T3, the number of patients with fixed space maintainers who had candidal colonization in both left and right lingual mucosa sites was also significantly higher in comparison to the number of patients with removable space maintainers (p < 0.05). When the changes in candidal colonization at each mucosal site was compared according

4.95 ± 2.935 T1: 4.66 ± 3.271, T2: 113.33 ± 26.240, T3: 123.59 ± 29.51; p < 0.001).

Oral Candidal Carriage

Candidal counts throughout the study are given in table 2. No statistical differences were observed in the candidal counts between T0 and T1 in either group (p > 0.05). At T2, group I showed significantly higher mean and total oral candidal counts in comparison to T0 (p < 0.05), whereas no significant changes were observed in group II. At T3, both groups showed significantly higher mean and total oral candidal counts in comparison to T0 (p < 0.05) (table 2).

The candidal colonization according to the mucosal sites is shown in table 3. When each oral site was examined separately, the candidal counts at the buccal sites in-

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to type of space maintainer, the increase in colonization numbers was found to be significantly greater for patients with fixed maintainers at the right and left buccal mucosa sites throughout the study ($p < 0.05$).

The correlation between the periodontal index scores and the candidal colonization at mucosal sites are given in table 4. In group I, no correlation was found between the plaque, gingival or bleeding on probing index measurements and the candidal counts at T0 or T1. However, positive correlations between all index scores and candidal counts were observed at T2 and T3.

In group II, positive correlations were observed between all index scores and candidal counts at T0 and T1 and between the plaque and bleeding on probing indices and the candidal counts at T2; however, no correlations were observed between the gingival index measurements and the candidal counts at T2 or between any of the periodontal index scores and the candidal counts at T3.

Discussion

The removable space maintainers did not affect plaque accumulation, although fixed space maintainers caused an increase in local plaque accumulation after 3 months of use. The difference between removable and fixed space maintainers could be due to the fact that the former can be removed during toothbrushing and thus does not impede plaque clearance. Because the gingival indices and bleeding on probing scores did not significantly differ between the regions with and without removable space maintainers, periodontal health was not affected by the use of removable space maintainers. The plaque scores did not significantly differ between T0 and T3 with fixed space maintainers, but the gingival index scores increased after T1 and the bleeding on probing scores at T2, and both continued to increase until T3. Because the gingival index and bleeding on probing scores are indications of periodontal health, the negative effect of the fixed space maintainers may therefore continue for 6 months. The lack of any negative effect (as shown by lower plaque scores) at T3 could be due to the Hawthorne effect, a phenomenon where subjects improve or modify their behavior when they know they are being observed [25]. It is therefore possible that the knowledge of a subsequent examination at 6 months as a part of this study prompted both the patients and their parents to be more proactive about oral hygiene activity, particularly for the sites where the space maintainers were used.

In contrast to the present study, similar increases in plaque accumulation and pocket depths for both removable and fixed space maintainers were previously reported [8], probably because of differences in the methodologies used. The earlier study had examined plaque scores of teeth that were banded or clasped over a defined time period; this could produce misleading results because overall increases in the index scores may result from poor oral hygiene unrelated to space maintainer use. However, the index scores in our study were obtained from teeth within the same region as the space maintainers, and these were compared with those in regions where no space maintainers were present.

The effects of removable and fixed orthodontic appliances on periodontal health have been studied previously. Although the patient age profiles and appliances used vary from those in our study, there were some similarities such as use of bands or acrylic bases that can be used for comparison. Arendorf and Addy [7] compared the plaque index scores of regions with and without appliances and observed that the plaque index scores increased where a removable orthodontic appliance was used; this contradicts our results. However, the authors stated that the increase was observed only on upper palatal sites and that most other dental sites showed a gradual decrease. Hence, the effect can be a result of inadequate brushing on palatal sites. Boyd and Baumrind [26] compared the periodontal status of bonded and banded molars before, during and after treatment with fixed orthodontic appliances. Corroborating our results, they reported that, during treatment, both maxillary- and mandibular-banded molars showed significantly greater gingival inflammation and plaque accumulation than bonded molars. These authors also observed that there was significantly more plaque accumulation and gingival inflammation in adolescents than in adults, thereby emphasizing the effect of age on oral hygiene status.

The observed increase in *E. faecalis* count during the use of both the removable and the fixed space maintainers in this study corroborates the findings of previous investigations that the oral status affects the presence of this microorganism [27, 28]. *E. faecalis*, a Gram-positive, facultatively anaerobic coccus causing normal human gastrointestinal infections, commonly causes secondary apical periodontitis [29]. Furthermore, accounting for up to 90% of all human enterococcal infections [30], it commonly causes nosocomial infections and has resistance to currently available antibiotics, thus representing a major health problem [27].

Souto and Colombo [28] reported a high prevalence of this species (80%) in subgingival biofilm samples collected from periodontitis patients and significant positive correlations between the presence of *E. faecalis* and clinical parameters of probing depth, clinical attachment level, bleeding on probing and plaque accumulation. Balaei-Gajan et al. [27] suggest that the positive associations between *E. faecalis* and the clinical indications of periodontal destruction and inflammation (pocket depth, clinical attachment, bleeding on probing and plaque index) are indications that this microorganism plays a role in the severity and/or progression of periodontitis. The findings of these previous studies therefore suggest that the presence of and/or increase in the *E. faecalis* population in the oral environment represents a potential risk for periodontal pathologies during the usage of fixed space maintainers.

We also examined whether the candidal carriage and extent of colonization were affected by space maintainer use; a significant increase in colonization and mean/total number of *Candida* among patients receiving treatment using fixed space maintainers was observed, whereas no significant changes in the total counts were observed among patients being treated using removable space maintainers. In addition, the changes in mean values were significant after 6 months of treatment. The effects of fixed space maintainers on candidal carriage has yet not been investigated; however, the results of this study corroborate those of previous studies reporting an increase in candidal counts with fixed appliances for orthodontic treatment [19]. In addition, Arendorf and Addy [7] reported an increase in candidal counts at 5 months of removable orthodontic appliance use, and considering that the counts further increased in regions where an appliance was being worn, they attributed the increase to the occlusive and protective effects of the appliance. However, they also noted that the candidal counts increased in regions where no appliance was present.

The findings of previous studies therefore suggest that the increase in candidal counts is attributable to poor oral hygiene. This hypothesis is supported by the findings of this study of multiple positive significant correlations between the candidal counts and the plaque, gingival and bleeding on probing index scores for both fixed and removable appliances. Furthermore, the positive correlation between the candidal counts and the index scores observed in the removable space maintainer group at T0 may also be another indication of a possible relationship between poor oral hygiene and candidal presence.

Considering the results of the present study and the above-mentioned data, further studies with higher sample sizes investigating the effects of space maintainers on the oral flora and oral health are needed since the small sample size is a limitation in the present study.

Conclusion

Both fixed and removable space maintainer use may result in an increase in the number of microorganisms in the oral cavity and in increases in the periodontal index scores. While using these appliances, patients should be closely monitored and informed that space maintainer use may increase the risk of dental caries and periodontal disease and that they must give special attention to their oral hygiene.

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