

Table S1. Literature review of studies focusing on the effect of fixed orthodontic appliances on oral microbiota, which were not included in the meta-analysis by Guo et al., 2017

Population	T*	Oral bacteria	Oral candida	Reference
N Mean/median age in years Type of sample PI/GI	(time of the sample collection)			
30 patients Age: 12 – 18 years Stimulated saliva samples	T0 = 0 T1 = 4 weeks T2 = 12 weeks	All samples were positive for <i>Neisseria</i> sp. Following changes were observed, but they were NS. <i>S. mitis/oralis</i> increased at T1, but decrease almost to the initial levels at T2 <i>S. anginosus</i> – a slight decrease at T1 <i>S. mutans</i> – decrease at T1 <i>S. salivarius</i> increased a bit at T2 <i>S. sanguinis</i> decrease at both times, T1 and T2 <i>S. parasanguinis</i> decrease at T1 and increase at T2 Coagulase – negative staphylococci decrease at T1 but increase at T2 <i>Staphylococcus aureus</i> decrease at T1 and T2	All samples were negative for <i>Candida albicans</i> .	(28)
17 patients Age: 11 – 30 years Oral rinses	T0 = 0 T1 = 2 weeks T2 = 6 weeks T3 = 12 weeks	NA	Increase of number of the colony forming units of the <i>Candida</i> sp. during orthodontic treatment (NS) Dominance of <i>C. albicans</i>	(11)
124 patients Mean age 19.5 years	T0 = 0 T1 = 1 month T2 = 6 months	NA	NS difference in the presence of <i>C. albicans</i> during treatment by an orthodontic appliance.	(17)

Oral swab	T3 = 1 year after treatment			
	T4 = 6 months after completion of ortho. treatment			
50 patients (case/control)	T = NA	Isolation of various <i>Streptococci</i> and <i>Enterococci</i> in patients treated with fixed appliances	Correlation between the duration of treatment by fixed appliances and frequency of <i>Candida</i> sp.	(8)
Age: 14 – 23 years		Low prevalence of live protozoans: <i>Trichomonas tenax</i> and <i>Entamoeba gingivalis</i>	Presence of <i>C. albicans</i> correlates with poor oral hygiene	
Oral swabs		Detection of <i>Acanthamoeba</i> sp. cyst in several patients treated with fixed appliances		
No statistically significant difference in PI in the group treated with fixed appliances				
15 patients	T0 = 0	Purple complex was significantly decreasing from T2	<i>Candida</i> sp. decreased significantly at T2 and the lowest value was at T3	(19)
Age: 11 – 41 years	T1 = 30 days	Green complex was at the lowest level at T3		
Nonstimulated saliva samples	T2 = 60 days	Yellow and orange complexes decreased significantly only at T3		
No statistically significant difference in PI and GI	T3 = 90 days	Red complex decreased significantly at T2 and the lowest value was at T3		
90 patients	T = NA	NA	Fixed orthodontic appliances did not enhance colonization of <i>Candida</i> sp.	(16)
Mean age 20.6 ± 7.1 years				
Oral swabs				
75 patients (3 groups, good oral	T0# ^{GOH} = 14.9 ± 7.9 months	<i>S. mutans</i> and <i>Lactobacilli</i> were detected in all patients	73.4% of the patients were positive for <i>Candida</i> sp. in	(9)

hygiene – GOH, poor oral hygiene (POH, POH and white spot lesion – POH/WSL, 25 patients per group)

T0#^{POH} = 13.4 ± 6.7 months

T0#^{POH/WSL} = 19.6 ± 11.3 months

Higher prevalence of *S. mutans* in patients with poor oral hygiene (NS)

Higher prevalence of *Lactobacilli*. A significant difference was observed between the GOH and POH/WSL groups

saliva and 60.9 % in dental plaque.

Significantly lower prevalence of *Candida* sp. in GOH than in POH and in POH/WSL was observed.

Mean age 14.4 ±1.5 years

Stimulated saliva and plaque samples

30 patients	T0 = 0	Significant increase of <i>S. mutans</i> and <i>Lactobacillus acidophilus</i> in T1 and continued up to T2 was observed	Total count of <i>Candida</i> sp. increased significantly during treatment by orthodontic appliances (starting from T1). <i>C. albicans</i> was the most predominant.
Age: 12 – 18 years	T1 = 6 weeks		
Saliva sample	T2 = 12 weeks		
	T3 = 18 weeks		

50 patients	T= 1,2,3,6 months before and after the beginning of treatment	NA	Significant increase of <i>Candida</i> sp. within 3 months after the installation of the orthodontic appliance (18)
Age 10 - 18 years			
Samples via gargle method			

60 patients	T0 = 0	A dramatic increase of <i>S. mutans</i> at T1 and T2	Increase of <i>Candida</i> sp. at T1 and T2 (5)
Age: 13 – 18 years	T1 = 2 months		
Plaque samples	T2 = 3 months		

60 patients	T0 = 0	NA	Significant increase of <i>Candida</i> at T1 in the group treated with fixed orthodontic appliance (15)
(28 patients with fixed orthodontic appliance and 32 with removable appliance)	T1 = 6 months		In the group treated with removable appliances, the increase of <i>Candida</i> was not significant
Age: 4 – 10 years			At T0, the most frequently isolated species was <i>C. albicans</i>

Oral swabs

At T1, the most frequently found species was *C. tropicalis*

95 patients (orthodontic group, 48 patients; control group, without appliances, 47 patients) Age: 12 - 16 years Stimulated saliva sample	T0 = 0 T1 = 6 months	Significant increase of <i>S. mutans</i> and <i>Lactobacillus</i> sp. at T1 in orthodontic group	NA	(10)
60 patients (orthodontic group, 30 patients; control group, without appliances, 30 patients) Age: 12 – 20 years Plaque samples Significant difference in PI in orthodontic and control subjects	T1 = 12.4 ± 6.2 months T2 = 3 years	Statistically significant greater prevalence of <i>Aggregatibacter actinomycetemcomitans</i> in the orthodontic group	NA	(13)

GI, gingival index; GOH, good oral hygiene; NA, not available; NS, non-significant; PI, plaque index; POH, poor oral hygiene; POH/WSL, poor oral hygiene and white spot lesion; ROA, removable orthodontic appliance; *T0, Before bonding of fixed orthodontic appliances; T1, T2, T3, After bonding of fixed orthodontic appliances

The mean wearing time at the time of samples collection; the orthodontic appliance was applied for a significantly longer time in the POH/WSL group than in other groups ($p = 0.017$)

Table S2. Correlations between the different taste preferences and changes in the oral environment of 30 patients

Deterioration of	PI	GI	percentage of 7 periodontal bacteria	percentage of 3 cariogenic bacteria	<i>Candida</i> sp.	<i>Candida</i> sp. and 7 periodontal bacteria
Favorite taste – sweet						
yes (n=13)	10 (77%)	7 (54%)	8 (62%)	6 (46%)	5 (39%)	10 (77%)
no (n=16)	8 (50%)	4 (25%)	8 (50%)	13 (81%)	0 (0%)	8 (50%)
<i>p</i> -value	0.249	0.143	0.711	0.064	0.011	0.249
Sweetening drinks						
yes (n=13)	8 (62%)	5 (39%)	6 (46%)	10 (77%)	3 (23%)	8 (62%)
no (n=17)	10 (59%)	6 (35%)	11 (65%)	10 (59%)	2 (12%)	11 (65%)
<i>p</i> -value	1.000	1.000	0.460	0.440	0.628	1.000
Alcoholic drinks						
yes (n=9)	5 (56%)	5 (56%)	5 (56%)	5 (56%)	1 (11%)	5 (56%)
no (n=21)	13 (62%)	6 (29%)	12 (57%)	15 (71%)	4 (19%)	14 (67%)
<i>p</i> -value	1.000	0.225	1.000	0.431	1.000	0.687
Energy drinks						
yes (n=9)	4 (44%)	1 (11%)	3 (33%)	8 (89%)	1 (11%)	4 (44%)
no (n=21)	14 (67%)	10 (48%)	14 (67%)	12 (57%)	4 (19%)	15 (71%)
<i>p</i> -value	0.418	0.100	0.123	0.204	1.000	0.225

GI, gingival index; PI, plaque index

Table S3. The percentages of 3 selected cariogenic and 7 periodontal bacteria relative to the total prokaryotic DNA at two time points – T0 (before bonding of fixed orthodontic appliances) and T1 (till the end of 7th month after bonding of fixed orthodontic appliances) in 30 patients

Selected bacteria	The difference in % of the total prokaryotic genome in GCF samples between T0 and T1 mean, median (5th, 95th PCTL)	p-value
<i>Streptococcus mutans</i>	0, 0 (-0.02, 0.01)	0.889
<i>Lactobacillus</i> sp.	0, 0 (-0.02, 0.01)	0.136
<i>Actinomyces</i> sp.	0.85, 0.44 (-1.10, 5.52)	0.027
All 3 cariogenic bacteria	0.84, 0.44 (-1.10, 5.45)	0.030
<i>Aggregatibacter actinomycetemcomitans</i>	-0.02, 0 (-0.04, 0.01)	0.866
<i>Porphyromonas gingivalis</i>	0, 0 (0, 0)	1.000
<i>Tannerella forsythia</i>	0, 0 (0, 0)	1.000
<i>Treponema denticola</i>	0, 0 (0, 0)	0.317
<i>Parvimonas micra</i>	0, 0 (0, 0)	0.317
<i>Prevotella intermedia</i>	0, 0 (0, 0)	0.314
<i>Fusobacterium nucleatum</i>	0.95, 0.13 (-2.98, 8.98)	0.339
All 7 periodontal bacteria	0.94, 0.13 (-2.98, 8.98)	0.329
All 10 bacteria	1.78, 0.47 (-2.04, 11.75)	0.086

T0, before bonding of fixed orthodontic appliances; GCF, gingival crevicular fluid; please note that when sampling GCF, dental plaque is sampled as well

p-value: The difference between T1 and T0 measurements is evaluated using the Wilcoxon matched-pairs signed-rank test.