

Effects of Orthodontic Appliances on Oral Microbiome and Periodontal Health

Mouna Benkhalifa*, Ines Dallel, Wiem Ben Amor, Samir Tobji, Adel Ben Amor

University of Monastir, Department of Orthodontics, Oral Health and Orofacial Rehabilitation Research laboratory, LR12ES11, 5000, Monastir, Tunisia

Corresponding Author*

Mouna Benkhalifa

University of Monastir, Department of Orthodontics, Oral Health and Orofacial Rehabilitation Research Laboratory, LR12ES11, 5000, Monastir, Tunisia

E-mail: mouna10mouna10@hotmail.com Tell: 21654619221

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Abstract

Introduction: Orthodontic treatment has an important role in enhancing esthetics, function, and self-esteem in patients. However, it may also be associated with the development of white spot lesions and gingival inflammation especially in patients with poor oral hygiene.

Objective: The purpose of this systematic review was to compare the effects of different orthodontic appliances on the oral microbiome.

Results: Regardless of the type of appliance, the orthodontic treatment caused qualitative and quantitative changes in the oral microbiome leading to an increase in the counts of cariogenic bacteria and periodontal pathogens.

Conclusion: The selected articles reported that, even though both aligners and fixed appliances result in dysbiosis of the oral microbiome, aligners have the most favorable effects on oral hygiene and periodontal health.

Keywords: Orthodontic appliances • Clear Aligners • Self-ligating brackets • Lingual brackets, Oral microbiome • Cariogenic bacteria • Periodontal pathogens

Introduction

The benefits of orthodontic treatment are numerous and, in most cases, the advantages outweigh the possible disadvantages. Orthodontic treatment can play an important role in enhancing esthetics, function, and self-esteem in patients [1]. However, it can also have some harmful effects on the teeth and periodontal tissues especially in patients with poor oral hygiene. It has been shown that fixed orthodontic treatment impedes oral hygiene procedures and induces specific changes in the oral environment such as decreased pH, biofilm accumulation, and increased levels of microorganisms in saliva and dental plaque.

Much progress has been achieved in orthodontics over the last decades particularly in terms of the quest to facilitate mechanics, obtain a more precise diagnosis and establish efficient treatment plans. Orthodontists, nowadays, have a large arsenal of sophisticated appliances at their disposal allowing them to treat effectively different types of malocclusions and to satisfy the diverse esthetic and functional demands of their patients.

In recent years, marketing companies have presented aligners and self-ligating brackets to overcome the adverse effects of conventional braces claiming that they are better for oral health. Considering that preserving the integrity of dental and periodontal tissues is one of the main concerns of orthodontists, the present systematic review was undertaken to analyze the effects of orthodontic appliances on the oral microbiome and to compare these effects according to the appliance type. This systematic review was structured by four main sections: Introduction, Materials and Methods, Results, and Discussion (IMRAD structure).

Materials and Methods

According to the PRISMA guidelines (Preferred Reporting Items for

Table 1: Search strategy.

Database	Search strategy
MEDLINE <i>via</i> PubMed -Cochrane Library	(Removable or fixed) Orthodontic appliance* OR aligner* OR lingual orthodontic appliance* OR self-ligating bracket*) AND (oral microbiota OR microbiological colonization OR cariogenic pathogen* periodontal pathogen* OR Streptococcus mutans OR Lactobacillus spp. OR Candida OR Tannerella forsythia OR Treponema denticola OR campylobacter rectus OR Fusobacterium nucleatum OR Aggregatibacter actinomycetemcomitans OR Prevotella intermedia OR Prevotella nigrescens OR Porphyromonas gingivalis)
ScienceDirect, Lilacs and BBO via VHL	Orthodontic appliances AND (oral microbiota OR microbiological colonization OR periodontal pathogens OR cariogenic pathogens)

Table 2: Study Selection Criteria.

Inclusion criteria	Exclusion criteria
Studies analyzing microbial samples collected from oral sites and orthodontic appliances.	Systematic reviews, retrospective studies, abstracts, author debates, summary articles.
Patients treated with multi-brackets appliances or clear aligners.	
No diagnosed systemic diseases.	<i>In vitro</i> or animal studies.
Standardization and training in oral hygiene.	

Table 3: Swedish council on technology assessment in health care criteria for grading assessed studies.

Grade A	High value of evidence. All criteria should be met: randomized clinical study or a prospective study with a well-defined control group, defined diagnosis and endpoints, diagnostic reliability tests, and reproducibility tests described blinded outcome assessment.
Grade B	Moderate value of evidence. All criteria should be met: cohort study or retrospective case series with defined control or reference group, defined diagnosis and endpoints, diagnostic reliability tests, and reproducibility tests described.
Grade C	Low value of evidence. One or more of the conditions below: large attrition, unclear diagnosis, and endpoints, poorly defined patient material.

Table 4: Classification of evidence level.

Level	Evidence	Definition
1	strong	At least two studies assessed with level 'A'
2	moderate	One study with level 'A' and at least two studies with level 'B'
3	limited	At least two studies with level 'B'
4	inconclusive	Fewer than two studies with level 'B'

Systematic reviews and Meta-Analyses), a precise question was formulated conforming to the PICO system. The acronym PICO stands for population, intervention, comparison, and outcomes which, for this systematic review, were defined as follows:

Population: Adolescents and adults.

Intervention: Orthodontic treatment with multi-brackets appliances and clear aligners.

Comparison: Between different orthodontic appliances.

Outcome: The effects of orthodontic treatment on the oral microbiome.

The present systematic review was undertaken to answer the following questions:

- What are the effects of different orthodontic appliances on the oral microbiome?
- Which orthodontic appliances are the best for oral hygiene and periodontal health?

Until December 2021, an electronic systematic search in the medical literature produced between 2010 and 2021, was performed to identify all papers reporting changes in the oral environment following the insertion of an orthodontic appliance.

To retrieve lists of peer-reviewed articles to be included in this systemic review, the search strategy illustrated in Table 1 was developed for the following databases: PubMed, Cochrane Trial Library, ScienceDirect, and Lilacs.

Mendeley was used for importing the research results, discarding duplicates, searching for available full-texts, and later for managing the citations. The first step in the article's selection process consisted of screening the titles and reading the abstracts of the pre-selected articles to exclude all irrelevant publications. The second step consisted of reading the full texts of the potentially relevant papers to determine if they met the eligibility criteria. Articles were selected based on the criteria listed in Table 2. Data extraction from the included articles was performed using a template similar to that of Lucchese et al. [2] which was modified for this review. Disagreements were resolved by discussions between the authors.

The methodological quality of the included studies was rated using a 3-point grading system described by the Swedish Council on Technology Assessment in Health Care Criteria for Grading Assessed Studies' (SBU) illustrated in Table 3.

This method was also used to assess the level of evidence of the conclusions drawn from this systematic review. Once a grade has been assigned to each study (A, B, or C), the review's level of evidence was determined according to the classification presented in Table 4.

Results

Global selection process

The literature research initially yielded a total of 576 publications. After removing duplicates only 484 articles remained.

After the title-screening process and reading through the abstracts of the remaining articles, only 59 were deemed useful and made it to the last phase which is reading the full texts if available and assessing their

relevance to the intended subject.

Only 55 full texts were available. The reading led to the exclusion of 10 of them. Hence, 45 articles were included in this review. The global selection process is illustrated in the PRISMA Flow Diagram following the PRISMA guidelines (Figure 1).

Data extraction

Data extraction from the included articles was performed using a template similar to that of Lucchese et al. [2] which was adapted to the necessities of our review.

Extracted data included first author, year of publication, sample size, age of participants, type of appliance, collection time of the microbial samples, collection methods, microbial analysis methods, microorganisms studied. All of the extracted data are listed in Table 5.

Quality analysis

According to the SBU grading system, the methodological quality of 2 of the included studies was high (grade A). Thirty-five studies were classified as grade B and we assigned a grade C to the remaining 8 articles. Hence, conclusions with a strong level of evidence could be drawn from this review. The most important source of bias was the absence of blinding procedures and the lack of clues about randomization.

Data synthesis

We included studies that evaluated and compared the effects of different orthodontic appliances on the oral microbiome. Certain studies analyzed samples obtained from oral sites such as salivary samples and dental plaque samples. While others analyzed the biofilm that formed on the surfaces of brackets, archwires, and aligners.

In the following tables (Tables 6-9), we exposed these effects according to the appliance type.

Discussion

The selected articles of this systematic review agreed that orthodontic treatment, regardless of the type of appliance, caused quantitative and qualitative changes in the oral microbiome leading to an increase in the counts of cariogenic bacteria and periodontal pathogens that are associated with dental caries and periodontal disease. However, there were significant variations between the different types of appliances depending on their plaque-retaining properties and removability.

Table 5: Characteristics of studies included in the review

Reference	Participants			Microbial analysis				quality
	Sample size	Age (years)	Groups/ appliance	sampling	Collection time	Analysis methods	Microorganisms studied	
Wang et al. 2019 [3]	15 subjects divided into 3 groups of 5	20-25	G1: Invisalign G2: Fixed appliance G3: control group	Salivary samples	T: after 6 months	High-throughput pyrosequencing based on the 16S rRNA gene	Salivary microbial communities (composition and function)	B
Zhao et al. 2020[4]	25	18 years or above	Invisalign	Unstimulated saliva samples	T0: before treatment T1: 6 months	Illumina MiSeq sequencing of the bacterial 16S rRNA.	Oral bacterial community	B
Mummolo et al. 2020[5]	80 subjects divided equally into 2 groups	G1: 20.4±1.7 G2: 21.3±1.7	G1: Invisalign (24M/16F) G2: SLB Damon Q2 (22M/18F)	Stimulated saliva collected by chewing paraffin tablet	T0: before treatment T1: 3 months T2: 6 months	CRT bacteria	<i>S mutans, Lactobacillus</i>	B
Mummolo et al. 2020[6]	90 subjects (56M/34F) divided equally into 3 groups	G1:21.5 G2:23.3 G3:18.2	G1: Invisalign G2: SLB Damon Q2 G3: removable positioner	Stimulated saliva collected by chewing paraffin tablet	T0: before treatment T1: 3 months T2: 6 months	CRT bacteria	<i>S mutans, Lactobacillus</i>	B

Guo et al. 2018 [7]	10 F	18-40	aligners	Subgingival plaque samples collected with sterilized periodontal curette.	T0: before treatment	16S rRNA gene sequencing	Subgingival microbial communities (composition and structure)	B
Sifakakis et al. 2018 [8]	30 subjects (13M/17F) divided equally into 2 groups	13.8	G1: aligners G2: SLB	Index teeth: first incisor and first molar Stimulated saliva collected by chewing paraffin gum for 5 min	T1: 1 month T2: 3 months T0: before treatment T1: 2 weeks T2: 1 month	High-throughput pyrosequencing Quantitative PCR	<i>S mutans, S sanguinis, Lactobacillus acidophilus</i>	B
Lombardo et al. 2013 [9]	27	G1: 21 G2: 14	G1: clear aligners 14 subjects (5M/9F) G2: fixed appliance 13 subjects (5M/8F)	Subgingival plaque from upper right first incisor and first molar	T0: before treatment T1: 1 month T2: 3 month T3: 6 month	PCR	Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Fusobacterium nucleatum, Treponema denticola and Campylobacter rectus	B
Demling et al. 2010 [10]	20 (6M/14F)	Dec-32	lingual brackets on the lower teeth only (the upper teeth are the control sites)	Gingival crevicular fluid (GCF) taken with sterile paper points. Index teeth: upper and lower first molar, first premolar and central incisor	T0: before bonding T1: 4 weeks	PCR	Aggregatibacter actinomycetemcomitans <i>P gingivalis</i>	B
Gujar et al. 2020 [11]	60 subjects divided equally into 3 groups	29-Nov	G1: CB G2: Invisalign G3: LB	Debonded brackets and rinsed aligners	T: 1 month after appliance placement	Cherckerboard DNA-DNA hybridization	Periodontopathogens	B
Lombardo et al. 2013 [12]	20 subjects divided equally into 2 groups	19-23	G1: CB with steel ligatures (2M/8F) G2: LB (3M/7F)	Stimulated saliva collected by chewing paraffin gum for 5 min	T0: before bonding T1: 4 weeks T2: 8 weeks	CFU counts	<i>S mutans, Lactobacillus</i>	B
Baka et al. 2013 [13]	20 M	14.2±1.5	G1: SLB in the maxillary right and mandibular left dentitions and CB in the opposite quadrants G2: vice versa (CB with steel ligatures) 3 types of brackets bonded on the 6 upper anterior teeth with a random distribution	Supra gingival plaque samples collected from the labial surfaces of the upper lateral incisors with sterilized curettes Non stimulated saliva	T0: before bonding T1: 3 months T0: before bonding	Dneasy blood and tissue kit (DNA extraction) + real-time PCR	<i>S mutan, S sobrinus</i> Lactobacillus casei, Lactobacillus acidophilus	B
Bergamo et al. 2017 [14]	20 (9M/11F)	15-Nov	1.active SLB 2.passive SLB 3.CB with elastomeric rings Upper right side: SLB	Debonded brackets GCF and subgingival plaque sampling by inserting a sterile paper strip in the gingival crevice of mesial and distal sides of upper canines	T0: before bonding T1: 1 month T2: 2 months T0: before bonding	Cherckerboard DNA-DNA hybridization	22 Bacterial species of the oral microbiome Total bacteria	B
Hassan et al. 2010 [15]	22 (10M/12F)	13-22	Upper left side: CB with steel ligature		T1: 1 week T2: 1 month T3: 3 months T4: 6 months	CFU counts under a stereomicroscope	<i>S mutans, Lactobacillus</i>	B
Al-Melh et al. 2020 [16]	80 subjects divided equally into 2 groups	G1: 26 G2: 17	G1: SLB 40 (8M/32F) G2: control 40 (11M/29F)	Stimulated saliva samples	T: after at least 12 months of treatment	PCR and real-time quantitative PCR	<i>S mutans, S sobrinus, S salivarius, S Gordonii, lactobacillus casei</i>	B
Ireland et al. 2019 [17]	24	14-Nov	SLB with molar bands and tubes to contralateral quadrants of the mouth with elastomeric ligature on one U2 bracket	-molars: supragingival plaque samples collected with curettes and subgingival plaque with sterile paper points	T0: before bonding	DNA was extracted with GeneElute PCR DNA Purification Kit.	periodontopathogens	B

				-U2: plaque adjacent to the bracket margins	T1: 3 months T2: before debonding T3: 3 months T4: 1 year	PCR/DGGE/ Microarray hybridization		
Jung et al. 2016 [18]	60 (21M/39F)	23.5	3 types of SLB brackets G1: Clarity-SL 22 subjects G2: Clippy-C 21 subjects G3: Damon Q 17 subjects	244 Debonded brackets (4 brackets per patient) of the upper and lower central incisors	At debonding after a treatment period of at least 12 months	Real-time PCR	Aggregatibacter actinomycetemcomitans Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, and Fusobacterium nucleatum	B
Jung et al. 2015 [19]	40 subjects divided equally into 2 groups	23.4	2 types of ceramic SLB brackets G1: Clarity-SL G2: Clippy-C	Debonded brackets of the upper and lower central incisors	At debonding after a treatment period of at least 12 months	Real-time PCR	Total bacteria <i>S mutans</i> <i>S sobrinus</i>	B
Do Nascimento et al. 2013 [20]	10 M		5 types of aesthetic brackets randomly bonded to the following teeth in each patient: lower left 3 4 5 6 and 7. -test groups: 3 types of SLB -control groups: 2 types of CB with elastics	Supragingival plaque samples 50 deboned brackets	T0: before bonding T1: day 21 T2: day 28	Culture/ CFU count Electron microscopy	 <i>S mutans</i>	A
Mummolo et al. 2013 [21]	60 subjects (27M/33F) divided equally into 3 groups	20.5	G1: CB with steel ligatures G2: SLB G3: control	Stimulated saliva samples	T0: before bonding T1: 3 months T2: 6 months	CRT bacteria test	<i>Smutans, Lactobacillus</i>	B
Pandis et al. 2010 [22]	32	13.6	G1: CB with elastomeric ligatures G2: SLB	Unstimulated saliva samples	T0: before bonding T1: 2-3 months	Culture/ CFU count	Salivary S mutans and total bacteria	B
Pejda et al. 2013 [23]	38 (13M/25F)	14.6±2	G1: CB with steel ligatures G2: SLB	Subgingival plaque samples collected with a sterile paper point.	T: 18 weeks	PCR (micro-Dent test)	Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, and Treponema denticola.	B
Uzuner et al. 2014 [24]	40 subjects (11M/29F) divided equally into 2 groups	14-16	G1: CB with steel ligatures G2: SLB	Stimulated saliva and plaque samples adjacent to the bracket margins of the lateral incisors	T0: before bonding T1: 1 month	Dentocult SM and LB kits	<i>S mutan</i>	B
Jing et al. 2019 [25]	15	14-20	G1: CB 6 subjects G2: SLB 9 subjects	Unstimulated whole saliva	T0: before treatment T1: 3 months T2: 6 months T3: 18 months	Quantitative PCR	Total bacteria <i>S mutans</i> <i>Lactobacilli</i>	B
Nalçacı et al. 2014 [26]	46 subjects divided equally into 2 groups	16-Nov	G1: CB with elastomeric ligatures (12M/ 11F) G2: SLB (10M/ 13F)	Plaque samples collected from the buccal surfaces of all bonded teeth	T0: before bonding T1: 1 week T2: 5 weeks	Colonies counted under a stereomicroscope	<i>candida</i>	B
Zheng et al. 2016 [27]	50	18-Oct	G1: CB	Microbiological samples collected via the gargle method	1 month 2 months 3 months 6 months Before and after bonding T0: before bonding	Culture/ identification of candida strains based on the color of colonies/ PCR	Aerobic and anaerobic bacteria	C
Akgun et al. 2014 [28]	(23M/ 27F) 13 (3M/10F)	16.2	2 groups of teeth: G1: 13 UL first premolar with Slide ligatures G2: 13 UL second premolar with conventional elastomeric ligatures	Supragingival and subgingival plaque samples obtained from the index teeth using a sterile curette (4 surfaces of each tooth)	T1: 1 week T2: 5 weeks	CFU counts		B

Andrucioli et al. 2012 [29]	18 (11M/7F)	29-Nov	CB	Debonded brackets. Each subject had 2 new brackets bonded to different PM in a randomized manner.	After 30 days of bonding	Checkerboard DNA-DNA hybridization	Oral microbiome	A
Dallel et al. 2019 [30]	101 (40M/61F)	G1: 19 ± 3.4 G2: 17 ± 3.8	G1: metallic/ceramic labial brackets 50 (20M/30F) G2: control 51 (20M/31F)	Salivary and supragingival plaque samples 12 Archwires and 50 debonded brackets	After a treatment duration of approximately 2 years	Gram staining, cultural and metabolic characteristics supplemented by API biochemical galleries (BioMerieux®, Paris, France)	Different bacterial species of the oral microbiome	C
Costa Lima et al. 2019[31]	6 patients 48 archwire fragments	18-30	CB with 4 different types of archwires: -Coated NiTi -Partially coated NiTi -Uncoated stainless steel -Uncoated NiTi	8 archwire fragments were collected from each patient (48 segments in total)	After 30 days of clinical use	Culture/ CFU counts	Total bacteria	B
Guo et al. 2016[32]	108 subjects 46 adults 62 children	Aug-32	CB	Subgingival plaque samples collected from lower central incisors and premolars	T0: before bonding T1: 1 month T2: 3 months	Quantitative real-time PCR	<i>P. gingivalis</i> , <i>F. nucleatum</i> , <i>P. intermedia</i> , and <i>T. forsythensis</i>	C
Guo et al. 2019[33]	10 F	18-40	CB	Subgingival plaque samples	T0: before bonding T1: 1 month T2: 3 months	16S rRNA gene sequencing	Microbial community of subgingival plaque	B
Jurela et al. 2013[34]	32	13-30	G1: CB 16 (6M/10F) G2: plastic brackets 16 (6M/10F) - bands with margins at the gingival margin OBM - bands with margins below the gingival margin OBSM	Stimulated saliva Subgingival plaque samples collected from:	T1: before bonding T2: 12 weeks	PCR/ cultivation/ CFU count	<i>S. mutans</i> , <i>S. sobrinus</i>	B
Klara Kim et al.2010 [35]	33	18-Dec	-brackets OBR	83 OBR 103 OBSM 54 OBM Index teeth: first molars and second premolars	T: after at least 6 months of treatment	Checkerboard DNA-DNA HYBRIDIZATION	<i>Subgingival microbiota</i>	B
Lara-Carrillo et al. 2010[36]	34 (14M/20F)	16.7±5.2	G1: CB Conventional brackets GA: at the beginning of treatment 28 (6M/22F) GB: at the end of treatment	Stimulated saliva obtained during 5 minutes by chewing an unflavored piece of wax Subgingival plaque samples from the lower incisors using a sterile dental curette	T0: before bonding T1: 1 month GA T0: before bonding T1: 1 month T2: 3 months GB T1: at debonding T2: 1 month T3: 3 months T4: 6 months	CFU counts Dentocult® SM Dentocult® LB Real-time qPCR	<i>S. mutans</i> , <i>Lactobacillus</i> <i>P. gingivalis</i>	C B
Liu H. et al. 2011[37]	48	29-Dec	20 (7M/13F)		T0: before bonding T1: 6 months T0: before bonding T1: 1 month T2: 2 months T3: 3 months	Dentocult® SM Dentocult® LB PCR	<i>S. mutans</i> , <i>Lactobacillus</i> 5 periodontal pathogens Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, and Treponema denticola	C
Maret et al. 2014 [38]	95	16-Dec	G1: CB 48 (16M/32F) G2: control 47 (23M/24F)	Stimulated saliva collected by chewing paraffin wax	T0: before bonding T1: 6 months T0: before bonding T1: 1 month T2: 2 months T3: 3 months	Dentocult® SM Dentocult® LB PCR	<i>S. mutans</i> , <i>Lactobacillus</i> 5 periodontal pathogens Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, and Treponema denticola	C
Montaldo et al. 2013[39]	19 (7M/12F)	22-Oct	CB	Subgingival plaque samples taken from the two upper second premolars	T1: 1 month T2: 2 months T3: 3 months	PCR	Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, and Treponema denticola	C

Pan S. et al. 2017 [40]	117	17-Nov	G1: CB with elastics 61 (22M/39F) G2: control 56 (16M/40F)	Subgingival plaque samples collected from the lower incisors using sterile paper points	T0: before bonding T1: 1 month T2: 2 months T3: 3 months T4: 6 months	PCR	<i>Porphyromonas gingivalis</i>	B
Peros et al. 2011 [41]	23	17-Dec	G1: CB with steel ligatures	Stimulated saliva samples	T0: before bonding T1: 6 weeks T2: 12 weeks T3: 18 weeks	Cultivation/ CFU counts	<i>S mutans, Lactobacillus</i>	B
Reichardt et al. 2019 [42]	10	15-Dec	CB with elastomeric ligatures	Supragingival plaque collected from UR first premolar and UR first molar using sterile standard cotton tips	T0: before bonding T1: 1 week	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry	<i>Supragingival microbiota</i>	B
Shukla et al. 2016 [43]	60	15-25	G1: CB	Plaque samples collected with a cotton swab from the labial and buccal aspects of anterior teeth and first molars	T0: before bonding T1: 2 months T2: 3 months	Dentocult® SM	<i>S mutans</i>	B
Shukla et al. 2017 [44]	60	13-18	CB	Plaque samples collected from buccal and labial aspects of the anterior teeth and four first molars using a sterile cotton swab	T0: before bonding T1: 2 months T2: 3 months	Dentocult SM kit Candida was cultured on Sabouraud's dextrose agar	<i>S mutans, candida species</i>	B
Pejda et al. 2013 [45]	22 (10M/12F)	18-30	GB	Salivary samples	T0: before bonding T1: 3 months	PCR	<i>S mutans, S sobrinus</i> Oral microbiota	B
Sun et al. 2018 [46]	50	G1: 12-30 G2: 12-33	G1: CB 30 (8M/22F) G2: control 20 (8M/12F)	Unstimulated saliva samples	T: after 10-12 months of bonding	PCR-DGGE and real-time PCR	<i>Pseudomonas and Streptococcus species</i>	C
Torlakovic et al. 2013 [47]	20 (8M/12F)	16-Oct	G1: CB	supragingival plaque samples collected from the labial surface of the maxillary central incisors	T0: before bonding T1: 4 weeks T2: 3 months T3: 5 months	Human Oral Microbe Identification Microarray (HOMIM)	periodontitis- and caries-associated bacteria	C

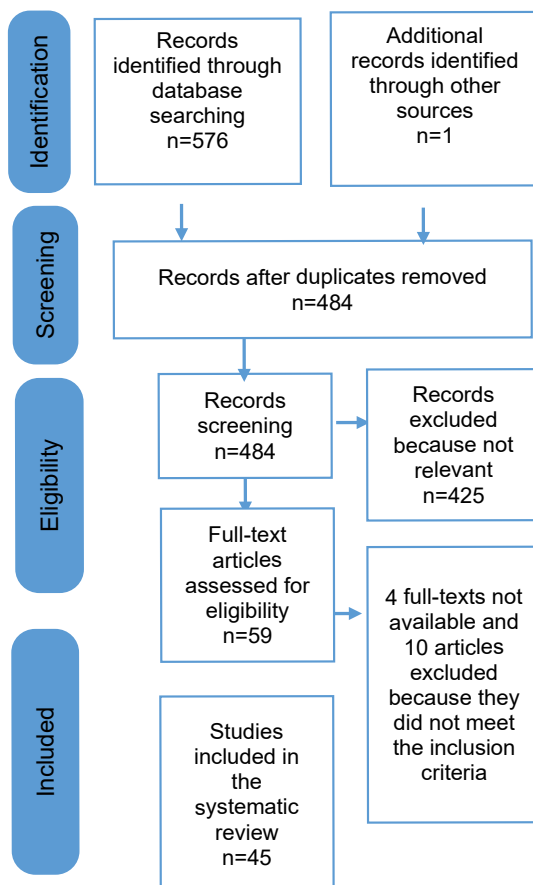


Figure 1: Articles selection process: PRISMA Flow Diagram.

Table 6: Labial fixed appliances.

Study	Objective	Results
Akgun et al. [28]	To compare the effects of a nonconventional elastomeric ligature (Slide®, Leone) with those of a conventional elastomeric ligature (Ormco, Orange) on microbial flora and periodontal status in orthodontic patients.	No significant differences between the two types of ligatures were evident at 1 week or 5 weeks after bonding concerning GI, PI, GBI, or PD scores ($P>0.05$). Similarly, aerobic and anaerobic bacteria count did not differ significantly on the teeth surface or the elastics ($P>0.05$).
Costa Lima et al. [31]	To compare the microorganism adhesion on coated, partially coated, and uncoated archwires after clinical use.	All the archwires presented microorganisms adhesion, with the Niti-coated group demonstrating the highest value ($p<0.001$).
Klara Kim et al. [35]	To compare the subgingival microbiota and clinical parameters in adolescents at sites treated with orthodontic bands or with brackets.	More bleeding on probing and deeper pocket depths were found around molar bands. The microbiological analysis revealed minor differences in the subgingival microbiota between bands and brackets.
Peros et al. [41]	To determine the physiologic changes of salivary flow rate, pH, and buffer capacity and the levels of SM and LB in patients undergoing fixed orthodontic treatment (Forestadent, Pforzheim, Germany).	A significant ($P < .05$) increase in stimulated salivary flow rate and salivary pH was found. The salivary levels of <i>S. mutans</i> and <i>Lactobacillus</i> spp also increased significantly ($P < .05$) and the major peak was at the third month of treatment followed by a decrease.
studies by Shukla et al. [43], [44]	To estimate counts and colonization pattern of <i>Streptococcus mutans</i> after application of fixed orthodontic appliances (0.22 MBT pre-adjusted Gemini stainless steel, 3M Unitek, CA, USA).	the levels of <i>S. mutans</i> increased significantly at the third month of orthodontic treatment with 90% of the patients showing severe colonization of S mutans.
Reichardt et al. [42]	To determine qualitative and quantitative microbiological changes that occur shortly after the implementation of fixed orthodontic treatment (Dentaurum, Ispringen, Germany).	As soon as one week after the insertion of fixed appliances, there was a significant increase in <i>Streptococcus</i> spp at the premolars and molars. In all individuals, symptoms of inflammation and gingivitis were detected as a response to the bacterial changes.
L. Guo [32], R. Guo [33],	To investigate changes in the subgingival microbial community at the early stage of fixed orthodontic treatment.	The amount of different periodontal pathogens including <i>P gingivalis</i> , <i>P intermedia</i> , <i>F nucleatum</i> , and <i>T denticola</i> , showed increasing trends during the first 3 months of treatment without significant differences.

Table 7: Self-ligating brackets (SLB).

Study	Objective	Results
Hassen et al. [15]	To evaluate the changes in microbial flora and periodontal status after orthodontic treatment with self-ligature versus archwire ligation techniques.	The use of conventional brackets with metallic ligatures led to a significant increase in the number of cariogenic bacteria, including <i>S. mutans</i> and <i>Lactobacillus</i> spp, compared to self-ligation brackets (0.022 in. Damon3™, Ormco, Orange, CA, USA), at different monitoring periods. These results are in agreement with the study conducted by Jing et al. [25]
Baka et al. [13]	To evaluate the effects of SLB (Damon Q; Ormco, Orange, Calif) and CB (Roth-equilibrium 2, 722-341; Dentaurum, Pforzheim, Germany) ligated with stainless steel ligatures on dental plaque retention and oral microflora.	The clinical parameters and the numbers of all microorganisms showed statistically significant increases from baseline to 3 months after bonding in both groups. The numbers of <i>S. mutans</i> and <i>L. acidophilus</i> were not statistically different between SLB and CB ligated with steel ligatures ($P>0.05$). These results are in agreement with the studies [22], [24], [26].
Mummolo et al. [21]	To investigate the microbial level of <i>S. mutans</i> and <i>Lactobacillus</i> during the conventional (Ovation GAC) and self-ligation (In-Ovation GAC self-ligating brackets) orthodontic treatment.	In the conventional bracket group, the percentage of patients with <i>S. mutans</i> colonization >10 CFU per milliliter of saliva reached a peak at 3 months (60%) followed by a decrease at 6 months of treatment (20%) while in the SLB group, this percentage continued to increase gradually during the monitoring period (25% at 3 months and 45% at 6 months). As for the <i>Lactobacillus</i> spp., their level showed a significant increase over time in the two treated groups compared to the control group.
Do Nascimento et al. [20]	To evaluate whether self-ligating brackets have an advantage over conventional brackets as determined by the adherence of S mutans. Five different types of aesthetic brackets were used.	Two experimental groups were active SLB (QuicKlear; Forestadent, Pforzheim, Germany ; and In-Ovation C; Dentsply GAC, Bohemia, NY); the other was a passive SLB (Damon 3; Ormco, Glendora, Calif). The two control groups were conventional brackets (Mystique; Dentsply GAC ; and Clarity; 3M Unitek, Monrovia, Calif). The results showed that the greatest numbers of SM colonies were found in an active self-ligating bracket group (In-Ovation C), and the fewest colonies were in a conventional bracket group (Clarity).
Bergamo et al. [14]	To assess whether the design of brackets influences the risk of developing periodontal disease.	the SLB InOvation®R (Dentsply GAC) and SmartClip™ (3M Unitek) presented the highest incidence percentages for the orange and red-complex bacteria (periodontal disease-associated pathogens) 2 months after bonding compared to the conventional brackets Gemini™ (3M Unitek).
Jung et al. [19]	To analyze the adhesion of <i>mutans streptococci</i> to two different types of self-ligating ceramic brackets (Clarity-SL and Clippy-C).	The adhesion of total bacteria and <i>S. mutans</i> to Clarity-SL braces was higher than that to Clippy-C braces ($p< 0.001$). Whereas, there was no significant difference in the adhesion of <i>S. sobrinus</i> between the two types of brackets.
Jung et al. [18]	To analyze the adhesion of periodontopathogens to three different types of SLB: two ceramic (Clarity-SL and Clippy-C) and one metallic (Damon Q).	<i>A. actinomycetemcomitans</i> , <i>P. gingivalis</i> , and <i>P. intermedia</i> adhered more to the Damon Q brackets in the mandibular teeth compared to the other two brackets.

Table 8: Lingual brackets.

Study	Objective	Results
Demling et al. [10]	To perform a preliminary study of the short-term effect of customized lingual orthodontic appliances (Incognito, Germany and Ibraces, Lingualcare, Dallas, Texas, USA) on periodontal and microbial parameters.	The percentage of <i>A. actinomycetemcomitans</i> on the bonded sites increased from 25% to 35% whereas the level of <i>P. gingivalis</i> (5%) did not change during the first week of treatment.
Gujar et al. [11]	To compare the microbial level changes in two different types of orthodontic appliances; labial fixed appliances and lingual fixed appliances.	Lingual fixed appliances (Ormco Corporation, Glendora, Calif), used for a month, showed more microbial contamination with periodontal pathogens including <i>F. nucleatum</i> , <i>P. gingivalis</i> , and <i>T. denticola</i> than labial fixed appliances (3M Unitek, Monrovia, Calif).
Lambardo et al. [12]	To compare the oral hygiene and caries risk of patients treated with labial and lingual fixed orthodontic appliances.	Patients wearing lingual orthodontic appliances (Ormco Corporation, Glendora, CA, USA) had more plaque retention, more gingival inflammation, and more <i>S. mutans</i> count 2 months after appliance placement compared to those treated with labial orthodontic appliance (American Orthodontics, Sheboygan, WI, USA).

Table 9: Clear aligner treatment (CAT).

Study	Objective	Results
Lombardo et al. [9]	To evaluate the subgingival microbiological changes during the first six months of orthodontic therapy with clear aligners (CA) (F22 Aligner, Sweden & Martina, Due Carrare, Padua) and fixed appliances (FA) (Primo Brackets, Sweden & Martina, Due Carrare, Padua).	The total bacterial load did not vary in the CA group, while a significant increase was detected after 3 and 6 months of treatment in the FA group. As for the individual bacterial species, <i>C. rectus</i> and <i>F. nucleatum</i> were often detected. Their levels remained stable in the CA group but increased progressively in the FA group.
Studies by Mummolo et al. [5], [6]	To investigate salivary levels of <i>S. mutans</i> and <i>Lactobacilli</i> , and other salivary indices in subjects wearing Invisalign® aligners in comparison with self-ligating brackets (Damon Q2, Ormco, Washington, DC, USA).	In the SLB group, the plaque index (PI) and the percentage of patients with risky salivary levels of <i>S. mutans</i> (CFU/ml>10) were significantly higher than those in the Invisalign group after 6 months of treatment.
Sifakakis et al. [8]	To assess the salivary prevalence of <i>S. mutans</i> , <i>Lactobacillus acidophilus</i> , and <i>S. sanguinis</i> among adolescents treated with thermoplastic aligners or SLB (In-Ovation R brackets GAC International).	There were no differences in the salivary levels of <i>S. mutans</i> and <i>Lactobacillus acidophilus</i> between patients treated for one month with aligners or with SLB. Whereas, lower levels of <i>S. sanguinis</i> were found in patients treated with aligners.
Wang et al. [3]	To investigate the changes in the oral microbiome in patients treated with the Invisalign system or with fixed appliances.	The Invisalign system did not show improved performance from the viewpoint of microbial composition and functional aspects of the oral microflora compared with fixed orthodontic appliances.

Ceramic brackets: metallic slot (Clarity)/ ceramic slot (Mystique)

Do Nascimento et al. [20] found that the lowest *S. mutans* colonization was verified with the Clarity brackets. This was explained by the fact that the ceramic slot of the Mystique bracket is porous with rough areas, and so it had greater potential for accumulating microorganisms compared with the smoother, less porous metallic slot of the Clarity bracket. According to this finding, it could be speculated that ceramic brackets are more inclined to bacterial colonization than metallic ones.

Self-Ligating Brackets (SLB)

The included studies in our review that compared the microbial effects of SLB with those of conventional brackets (CB) found different results shown in Table 7. Certain authors [15, 25, 26] agreed on the fact that SLBs have advantages over CBs because they eliminate the use of ligatures, reduce friction, exert lower forces on the teeth and allow for better oral hygiene maintenance. While others [22,24] found no significant differences between these two types of brackets. This finding could be explained by different reasons. The first is that the calcification of dental plaque may lead to obstacles in the functioning of the opening and closing mechanism of SLBs. Second, the components of SLBs are not subjected to regular renewal such is the case of ligatures. And third, the use of elastomeric chains or other auxiliaries with SLBs might cancel out their benefits.

Jung et al. [18] assessed the adhesion of total bacteria and periodontopathogens to three different types of self-ligating brackets: two ceramic (Clarity-SL and Clippy-C) and one metallic (Damon Q). The clarity-SL bracket is larger and has a more complex design; instead of having a cap that obstructs the slot, it is a slot-opened bracket with additional NiTi clips at both ends which may provide suitable niches for bacterial accumulation. As for the Damon Q bracket, it is made of stainless steel which has a greater plaque-retaining capacity than ceramic owing to its high critical surface tension and total work of adhesion. Based on these assessments, we can conclude that the bacterial adhesion to orthodontic devices is affected by the characteristics of their surfaces as well as their size and design. The location of the bracket whether it is on the upper or lower teeth is another factor that needs to be considered. Jung et al.

[18] showed that all the tested bacteria showed greater adhesion to the brackets of mandibular incisors. This finding may be explained by the location of the sublingual salivary gland duct near the lower incisors and by the reduced inter-bracket distance which may facilitate plaque retention. However, in another study also by Jung et al. [19], it was revealed that the adhesion of *S. mutans* was greater to the maxillary teeth. This can partly explain why white spot lesions are more frequent on the upper incisors than the lower ones.

Clear Aligners Treatment (CAT)

The included studies in our review [3,6,8] reported that, even though both aligners and fixed appliances resulted in dysbiosis of the oral microbiome, aligners had the most favorable effects on oral hygiene and periodontal health. This can be explained by different reasons. First, aligners are removable allowing patients to maintain their oral hygiene without the interference of brackets and wires. Secondly, each pair of aligners are changed almost every two weeks thus, the biofilm lingering for aligners is less than that of fixed appliances. And third, patients treated with aligners display better compliance with oral hygiene.

Despite these benefits, CAT can induce changes in the oral microbiome as revealed in the study conducted by [7]. These microbial changes could be explained by the fact that Aligners are worn almost all day long, they cover all tooth surfaces and their margins overlap the marginal gingiva thus, they impede the self-cleaning by saliva and may cause plaque accumulation. Also, the use of bonded attachments might provide additional plaque-retaining surfaces on the teeth.

Lingual brackets

Studies included in our review [10,12] revealed that the most consequent side effects on the oral microbiome and periodontal health have been occasioned by lingual appliances because plaque deposits on the lingual aspects of teeth are more difficult to remove with standard oral hygiene procedures compared to labial and buccal surfaces.

Conclusions

- Conventional brackets can be used for all types of patients.

However, they are not the appliance of choice for treating patients with severe periodontitis.

- Aligners should be preferred over fixed appliances in periodontally compromised patients because they are removable and allow the maintenance of better oral hygiene.
- Self-ligating brackets are capable of exerting lower force levels providing more favorable periodontal reactions in patients with previous bone loss. However, the use of elastomeric chains should be avoided as much as possible with this type of brackets because they facilitate plaque accumulation and induce friction.
- Lingual brackets are not recommended in patients with poor oral hygiene. They also might cause tongue irritations so the patient must be informed of this beforehand.
- Patients' motivation for oral hygiene is more than enough to counteract or even avoid the microbial imbalance caused by orthodontic treatment.

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