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Review

Dynamics of the oral microbiome during orthodontic treatment and antimicrobial advances for orthodontic appliances

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SUMMARY

The oral microbiome plays an important role in human health, and an imbalance of the oral microbiome could lead to oral and systemic diseases. Orthodontic treatment is an effective method to correct malocclusion. However, it is associated with many adverse effects, including white spot lesions, caries, gingivitis, periodontitis, halitosis, and even some systematic diseases. Undoubtedly, increased difficulty in oral hygiene maintenance and oral microbial disturbances are the main factors in developing these adverse effects. The present article briefly illustrates the characteristics of different ecological niches (including saliva, soft tissue surfaces of the oral mucosa, and hard tissue surfaces of the teeth) inhabited by oral microorganisms. According to the investigations conducted since 2014, we comprehensively elucidate the alterations of the oral microbiome in saliva, dental plaque, and other ecological niches after the introduction of orthodontic appliances. Finally, we provide a detailed review of recent advances in the antimicrobial properties of different orthodontic appliances. This article will provide researchers with a profound understanding of the underlying mechanisms of the effects of orthodontic appliances on human health and provide direction for further research on the antimicrobial properties of orthodontic appliances.

INTRODUCTION

The exploration of oral microbiomes dates back more than 300 years. In 1670, bacteria in dental plaque samples were visually observed directly by Antonie van Leeuwenhoek using his self-designed microscope, marking the discovery of the oral microbiome.[1–3] Initially, the research of the oral microbiome was dependent on time-consuming and painstaking techniques of the isolation and cultivation of individual strains. With advances in scientific techniques such as microscopy and genomics, new microbial detection techniques are emerging, encompassing quantitative real-time PCR (qPCR),[4] checkerboard DNA-DNA hybridization,[5] 16S rRNA sequencing,[6] fluorescence *in situ* hybridization-based microscopy (FISH-based microscopy),[7] metagenomics,[8] metatranscriptomics,[9] and more. These new technologies allow for faster and more comprehensive analysis of complex and diverse microbiomes and have greatly improved the level of resolution in oral microbiome research, leading to a more profound understanding of oral microbiome.[2,10]

The oral microbiome, the collective genome of the microbes, is the second largest microbiome after the gut and is highly diverse.[11–13] According to statistics, the human oral microbiome database (HODM) provides comprehensive information on approximately 700 prokaryotic species residing in the human oral cavity, which are mainly from seven phyla: Actinomycetota, Bacteroidota, Bacillota, Fusobacteriota, Pseudomonadota, Saccharibacteria, and Spirochaetota.[1,14,15] These oral microbes play a key role in human oral and systemic health and disease through polymicrobial synergy and dysbiosis and host-community interactions.[13] For example, Pozhitkov et al. found different oral microbiome signatures in subjects with periodontitis, edentulism, or oral health, with patients with periodontitis exhibiting the greatest diversity in their oral microbiota, in which 29 bacteria species displayed a significantly higher abundance compared to that in other subjects.[16] Recently, more systemic diseases, such as inflammatory bowel disease, other gastrointestinal diseases,[17,18] diabetes,[19,20] and cardiovascular disease,[21] have been proven to be associated with the oral microbiota.

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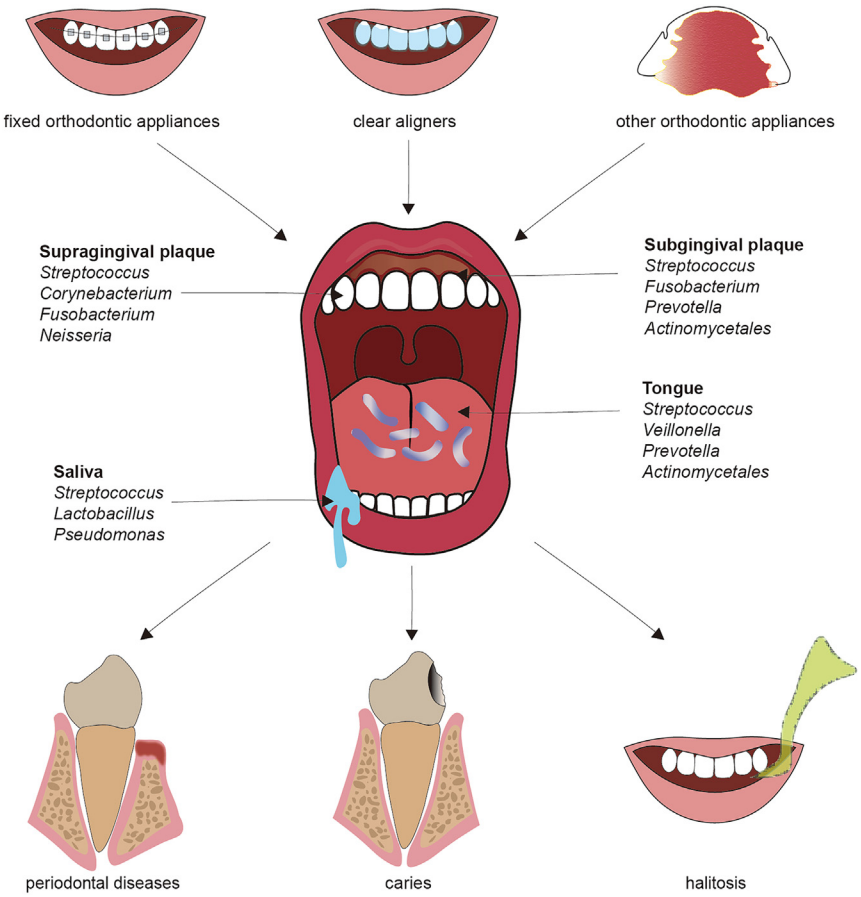

Figure 1. The figure illustrates that different types of orthodontic appliances placed in the oral cavity cause changes in the oral microbiota at different ecological sites in the oral cavity, which may increase the possibility of dental caries, periodontitis, and halitosis

Malocclusion, with a global prevalence of 56%, is one of the three most common diseases observed in oral clinical practice. It not only seriously affects patients' facial aesthetics, but also affects patients' mental health and stomatognathic system function.[22] Orthodontic treatment is an effective method to correct malocclusion. With the development of society and the economy, people's demand for aesthetic dentistry is increasing, especially clinical orthodontic treatment.[23] However, orthodontic treatment is associated with many adverse effects, including white spot lesions (WSLs),[24] caries, gingivitis,[25] periodontitis,[26,27] and halitosis.[28]

There is no doubt that increased difficulty in oral hygiene maintenance and microbial disturbances are the main factors in the occurrence and development of these adverse effects. Meanwhile, some researchers also have reported the alteration of the oral microbiota and disruption of the oral ecological balance during orthodontic treatment with either fixed appliances (FAs) or clear aligners (CAs). As early as 1983, Mattingly et al. investigated the relationship between orthodontic treatment and the oral microbiota, confirming that direct bonding of fixed orthodontic appliances enhanced colonization by *Streptococcus* (*S.*) *mutans*,[29] which bright a new perspective to explore the microbial etiological mechanisms and treatment approaches of adverse changes caused by orthodontic treatment.

In recent years, with the development of materials science and orthodontic appliance processing, numerous researches have been conducted to improve the antimicrobial properties of orthodontic appliances. The antimicrobial materials in the field of orthodontics are divided into three major classifications: metals and metal compounds, inorganic nonmetallic compounds, and organic compounds. In addition, there are two processing strategies to improve the antimicrobial properties of orthodontic appliances. For one strategy, antimicrobial materials are incorporated into orthodontic adhesives or acrylic resins, and for another, antimicrobial materials are coated on the surface of different orthodontic appliances.

Under this circumstance, this review intends to introduce the distinct ecological environments in the mouth and the effects of oral microbiota on oral health and diseases. According to the investigations since 2014, we also comprehensively elucidated and analyzed the alterations of the oral microbiome at different ecological niches (including saliva, supragingival plaque, subgingival plaque, and others) after the introduction of orthodontic appliances (Figure 1). On this basis, this present work provides a detailed review of the recent progress in the antimicrobial properties of different orthodontic appliances. The antimicrobial mechanisms, applications, and biocompatibility of each antimicrobial material classification are thoroughly introduced. We hope that this review will provide researchers with a profound insight into the microscopic mechanisms of the effects of orthodontic appliances on human health, and provide guidance and direction for further advances in the antimicrobial properties of orthodontic appliances and the oral hygiene maintenance in orthodontic patients.

SITE SPECIALIZATION OF THE ORAL MICROBIOME

The human oral cavity contains different habitats, including saliva, soft tissue surfaces of the oral mucosa, and hard tissue surfaces of the teeth (including supragingival plaque, and subgingival plaque), which provide nutrients and exquisite unique colonization spaces for oral microorganisms (Figure 2).[13,30] With the progress of microbiological analysis techniques, distinct microbial communities have been observed in different

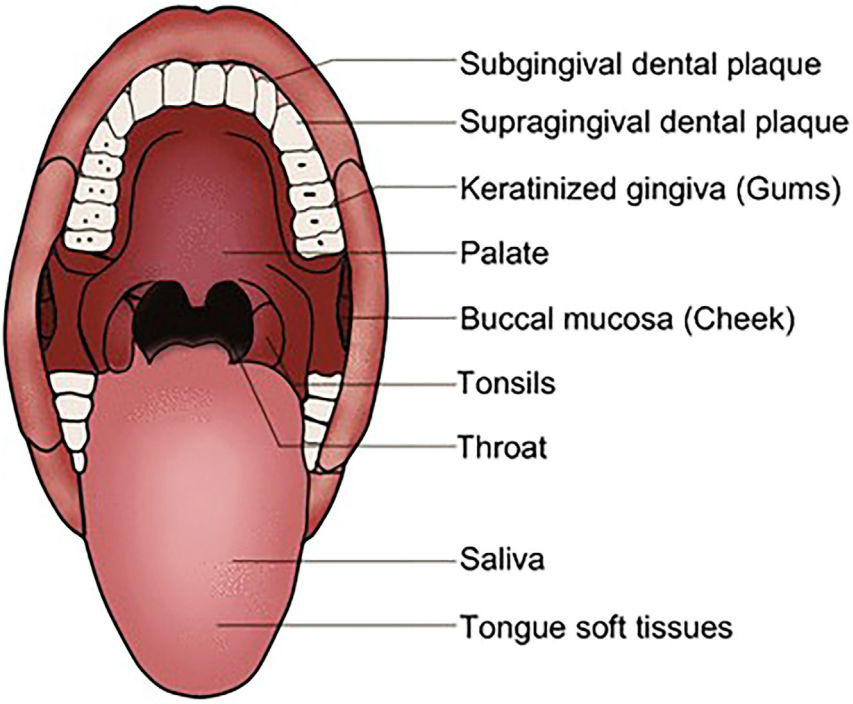

Figure 2. Major habitats within the mouth including saliva, soft tissue surfaces of the oral mucosa (buccal mucosa, keratinized gingiva, palate, tonsils, throat, and tongue soft tissues), and hard tissue surfaces of the teeth (supragingival and subgingival dental plaque (tooth biofilm above and below the gum)

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oral niches, which can also be called site specialization.[2] Here, this present review focuses on the environmental characteristics and microbial colonization features of different niches.

Saliva

Salivary plays a key role in maintaining oral health, which is reflected in various aspects. Saliva lubricates the mouth, facilitates chewing and swallowing of food, buffers pH to neutral to protect teeth from demineralization, and calcium and phosphorus ions in saliva contribute to the remineralization of teeth hard tissue. The high accessibility of saliva has led to its widespread application in biomarker research, such as head and neck squamous cell carcinomas, chronic periodontitis, and dental caries.[31] In addition, the salivary microbiota is critical to the maintenance of oral health.

Salivary microbes come mainly from the shedding of biofilms from the surfaces of human oral tissues, especially the tongue. It is estimated that there are approximately 108 viable microorganisms per milliliter of saliva.[32,33] Saliva samples were collected by Keijser et al.[34] from 71 healthy adults and sequenced through the Genome Sequencer 20 system at 454 Life Sciences. There are 3621 species-level phylotypes tested in saliva. Among them, Firmicutes and Bacteroidetes were the predominant phyla. Some microorganisms in saliva can be biomarkers of dental caries, a common multifactorial infectious disease. *S. mutans* has been demonstrated to be a central pathogen for caries[35] and other species *S. sobrinus* may also play a role. *Lactobacillus* is also considered as a significant facilitator of dental caries[36] and *Actinomycetes* have also been shown to contribute to the onset and development of root surface caries.[37] Hyposalivation is a significant risk indicator for the progression of dental caries.[38]

Soft tissue surfaces of the oral mucosa

Oral mucosa is the stratified squamous epithelium in the mouth, which is mainly divided into masticatory mucosa, lining mucosa, and specialized mucosa, including buccal mucosa, soft palate, hard palate, gingiva, tongue dorsal mucosa, and so on. As a result of the continuous shedding of oral mucosal epithelial cells, accompanied by oral microbiota, swallowing, and salivary flushing, the development of thick biofilms is avoided. However, it has recently been demonstrated that the epithelial surface of the tongue dorsal provides a potential for the formation of thick biofilms due to the abundance of papillae and the complex structural environment of the epithelial surface.[30]

Microorganisms on the soft tissue surface of the oral mucosa are associated closely with oral health and disease. Halitosis, oral candidiasis, and oral cancer have been shown to be associated with disorders of microorganisms on the oral mucosal surface. And ulcerated or diseased mucosa has also been confirmed to typically have larger numbers of microorganisms that accumulate and infiltrate the tissue.[1]

Hard tissue surfaces of the teeth

Hard tissue surfaces of the teeth provide a unique and significantly distinct setting for the colonization of the oral microbiome.[13] Plaque is a complex community of bacteria adhering to the surface of the teeth, embedded in a polymeric matrix derived from the host and microbiome, and has been categorized as supragingival plaque and subgingival plaque. The supragingival plaque is distributed above the gingival margin and is usually composed of a variety of microorganisms, including *Streptococci*, *Actinomycetes*, *Lactobacilli*, and so on. These bacteria are associated with the development of caries and the formation of supragingival calculus.[39]

Non-adherent subgingival plaque is directly in touch with the binding epithelium and the gingival sulcus epithelium and is mainly composed of gram-negative anaerobic bacteria, such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Fusobacterium nucleatum* and so on. These bacteria have a close relationship with the inflammation of the periodontal tissues.[39] Socransky et al.[40] divided the microorganisms in subgingival plaque into five complexes. The first complex consisted of *Bacteroides*

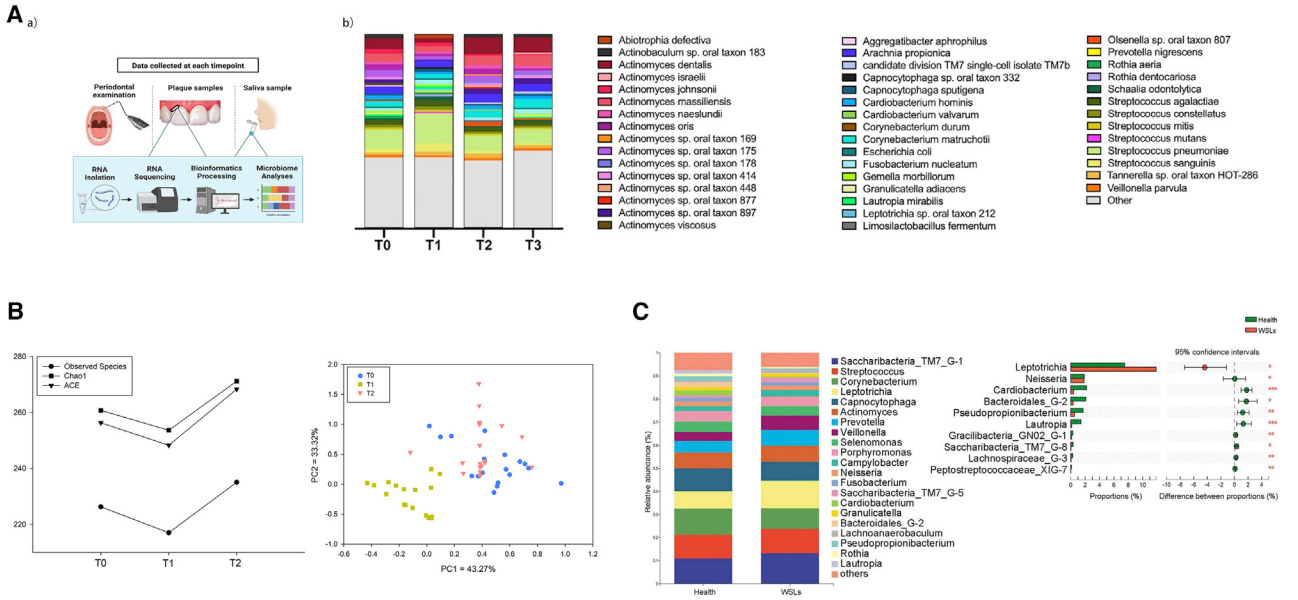

Figure 3. Schematic illustrations of alterations of the oral microbiome in orthodontic patients with fixed appliances

(A) (a) Overview of study design illustrating the visit events at each time point during 3months orthodontics; (b) Plaque species taxonomic profiles by visit (T0, baseline, day of bonding; T1, 1-week postbonding; T2, 6-week postbonding; T3, 12-week postbonding).

(B) Comparison of salivary microbial diversity before (T0) and 3 months (T1) and 6 months (T2) after treatment.

(C) Comparison of microbial composition in supragingival plaque between white spot lesions (WSLs) and health group after 6–12 months orthodontic treatment. Adapted with permission from.[48–50] Copyright 2024, SAGE Publications.

forsythus, *Porphvromonas gingivalis,* and *Treponema denticola*, which were named "red complex" and were intensively associated with the onset and progression of periodontitis.

ALTERATIONS OF THE ORAL MICROBIOME IN ORTHODONTIC PATIENTS WITH FIXED APPLIANCES

Fixed appliances (FAs), commonly known as dental braces, are orthodontic devices that cannot be removed by the patients and are designed to align teeth and correct bites. They consist of brackets or bands, which are bonded to the teeth, and an archwire that connects the brackets or bands, exerting pressure on the teeth to gradually move them into the desired position. Moreover, with the increasing demand for aesthetics, comfort, and convenience, FAs have derived different types that can be categorized according to their material (metal, ceramic, or plastic) and their position on the teeth (labial or lingual). FAs enable comprehensive control of three-dimensional tooth movement, making the treatment outcome more predictable, and therefore versatile for a wide range of complex orthodontic cases.

However, FAs are a potential risk factor for an increased accumulation of dental plaque because the rough bracket and band surfaces, or archwires complicate oral hygiene maintenance, which has been proven to contribute to the development of WSLs, gingivitis, and subsequently cavitated lesions and periodontitis.[41–44] It has been demonstrated that individuals treated with FAs for 1 year have a significantly higher prevalence and incremental increase in active carious lesions compared with those without FAs[45]; and the longer the duration of FA orthodontic treatment, the higher the prevalence/extent of active carious lesions.[46] Additionally, Ristic et al. also observed that adolescents treated with FAs may transiently increase all periodontal indices and stimulate the growth of periodontopathogenic bacteria, but have no destructive effect on deep periodontal tissues.[47]

Obviously, the change in the oral microbiome is inextricably related to the detrimental effects of FAs. In the last decade, abundant studies have been conducted to explore the influence of traditional FAs on oral microbiota, which will be elaborated upon (Figure 3).

Effect of fixed appliances on saliva microbiome

Among the several different ecological niches of the human oral cavity, saliva is relatively easy to collect and contains a significant abundance of microorganisms shed from the surface of the oral soft tissues.[10] As a result, saliva is a preferred choice in studies of microbial changes in the oral cavity (Table 1).[1,51–54]

FA: fixed appliances; OHI-S: simplified oral hygiene index; PCR: polymerase chain reaction; GOH: good oral hygiene; POH: poor oral hygiene; POH/WSL: poor oral hygiene with white spot lesions; CFU: colony forming unit; DMFT: decayed, missing and filled teeth index; SM: Streptococcus mutans; LB: Lactobacillus; WSL: white spot lesions; CB: conventional bracket; SLB: self-ligating bracket; sIgA: secretory immunoglobulin A; MPO: myeloperoxidase; LDH: lactate dehydrogenase; GI: gingival index; PLI: plque index; PD: probing depth; PCR-DGGE: polymerase chain reaction-denaturing gradient gel electrophoresis; qPCR: quantitative real-time PCR; BOP: bleeding on probing; MGI: the modified gingival index; GBI: gingival bleeding index; IL-1β: Interleukin-1 beta; MIF: macrophage migration inhibitory factor.

After being inserted for a short period of 3 months, FAs cannot induce significant cariogenic alterations of the oral microbiome in saliva. For example, one research evaluated levels of the cariogenic bacteria *S. mutans* and *S. sobrinus* in the saliva of patients after 12 weeks of FA treatment. The results displayed that the FAs did not induce significant alterations of the cariogenic bacteria in saliva in the early stage.[55] *S. mutans* and *S. sobrinus*, a specific type of acid-producing bacteria, is known to colonize tooth surfaces and potentially damage hard tooth structures in the presence of fermentable carbohydrates.[66,67] Additionally, intended to explore the changes in the salivary microbiota FA treatment, Zhao et al.[49] conducted a 6-month longitudinal study by 16S rRNA gene high-throughput sequencing and obtained similar results, which demonstrated a significantly decrease of microbial diversity after 3 months of treatment. However, the dynamic alteration in species did not induce the deterioration of oral health (Figure 3B). In contrast, Klaus et al.[56] collected saliva samples from 3 groups (good oral hygiene (GOH), poor oral hygiene (POH), and poor oral hygiene with WSLs (POH/WSL)) of 25 patients undergoing active FA appliance treatment in both jaws for at least 3 months. The investigation showed there was a high carriage of *Candida species* (spp.), *S. mutans*, and *Lactobacilli* in oral microbiome of all patients with orthodontic treatment, especially patients with WSLs. Different outcomes may be attributed to the absence of a pre-treatment control group.

Unfortunately, the long-term presence of FA in the oral cavity can facilitate an increase in the caries microflora. An experiment was conducted to assess the salivary flow rate and pH and oral microbes in patients before starting FA treatment and after 6, 12, and 18 weeks of treatment. Selective media was applied for the isolation and colony counts of *Candida albicans*, *S. mutans,* and *Lactobacillus acidophilus*, which were demonstrated to increase significantly during orthodontic treatment. Meanwhile, the research also proved that there was a significant decrease in salivary pH and no significant change in salivary flow during the treatment.[57] *Candida albicans* is aciduric, can enhance the cariogenic potential of *S. mutans* biofilms, ferments dietary sugars, and produces enzymes that degrade collagen, which has been demonstrated to play an important role in the development of dental caries.[68] *Lactobacillus acidophilus* also plays an essential role in the progression of dental caries.[69] Similarly, another study recorded the salivary pH, buffer capacity, *S. mutans,* and *Lactobacillus* spp. counts in patients at 0 and 6 months after FA treatment. The results showed a significant increase in *S. mutans* and *Lactobacillus* spp. values during the follow-up and significant change of the salivary pH, and buffer capacity.[69] Employing 16S rRNA sequencing and qPCR, Jing et al.[60] compared variations in salivary microorganisms between the conventional brace group and the self-ligating brace group during 18 months of treatment and found that S. mutans in patients in conventional brace group increased significantly in the late period of treatment, which revealed that patients are susceptible for WSL after long-term orthodontic treatment, especially patients with conventional braces. Jing et al.[60] also investigated the changes in some salivary parameters, including secretory immunoglobulin A (sIgA), myeloperoxidase (MPO), and lactate dehydrogenase (LDH). These parameters remained constant during treatment and no correlation was detected between sIgA and salivary microbiome.

Furthermore, differences in salivary properties and salivary microorganisms prior to orthodontic treatment can lead to differences in patient dental and periodontal health during treatment. Catunda et al.[59] explored whether differences in pre-treatment salivary Stephan curve kinetics and salivary microbiome characteristics were associated with the development of WSL in FA orthodontic patients. The findings revealed no significant differences in salivary microbiome richness, Shannon alpha diversity, and beta diversity between the two groups. However, *Capnocytophaga sputigena* and *Prevotella melaninogenica* were predominantly found in patients with WSL, whereas *S. australis* was negatively associated with the occurrence of WSL. Through analyzing the salivary Stephan curve kinetics, the change in salivary pH at 5 min was found to be related to the abundance of acid-producing bacteria in saliva.

FAs can contribute to increased levels of periodontal disease-associated microorganisms within a short period of time. Recently and innovatively, Liu et al.[61] analyzed alterations in the oral salivary, supragingival plaque, and intestinal microbiome of patients after 1 month of orthodontic FA treatment by applying metagenomic sequencing. The research demonstrated that *Fusobacterium, Aggregatibacter, Cardiobacterium, and Actinobacillus* elevated in the saliva at the genus level after wearing FAs for 1 month. And at the species level, periodontal disease-associated pathogens including *Aggregatibacter actinomycetemcomitans* and *Cardiobacterium hominis* increased and the relative abundance of probiotic *Faecalibacterium prausnitzii* significantly dedcreased in the saliva. The oral salivary microbiome develops a detrimental change in periodontal health during the initial phase of orthodontic appliance placement. In addition, the alterations in intestinal microbiota were also observed after one month of orthodontic treatment. Additionally, AlShahrani et al.[62] compared the salivary microbial distribution between two groups wearing FAs for more than 3 months: high-altitude dwellers and sea-level controls. The outcome demonstrated that the microbial homeostasis was perturbed because of the presence of biomaterial in the form of FAs. Alterations in the salivary microbial equilibrium may qualitatively represent a direct risk factor for periodontal disease due to an increase of periodontopathogenic species in the saliva. Meanwhile, exposure to high altitudes has exacerbated the dysbiosis of salivary microbiota equilibrium.

In addition, long-term FA orthodontic treatment disturbs the oral salivary microbial ecological equilibrium, which is a risk factor for promoting the transition from oral health to periodontitis. A study collected saliva samples and supragingival plaques of 71 patients with FAs before placement (T0), six months after placement (T1), and then when appliance removal (T2). The saliva microbiome was analyzed by 16S rRNA meta-sequencing. At the phylum level, the findings indicated a significant increase in Bacteroidetes and Saccharibacteria (formally TM7) and a decrease in Proteobacteria and Actinobacteria over time, and the genus level analysis demonstrated that there was a significant increase in anaerobic and facultative anaerobes in both plaque and saliva, which could draw a conclusion that the oral microbiome dysbiosis induced by FAs potentially represented a transitional stage in

Table 1. A summary of the researches on the effect of fixed appliances on oral microbiome in saliva

Appliances	Groups	Collection samples	Time points	Microbial detection techniques	Related parameters	Reference
FA	FA group	Saliva	T0 (baseline), T1 (12 weeks)	PCR (*Streptococcus mutans* and *Streptococcus sobrinus* counts)	Stimulated saliva flow, OHI-S, DMFT	Sudarević et al.[55]
FA	FA group	Saliva	T0 (baseline), T1 (3 months), T2 (6 months)	16S rRNA sequencing	–	Zhao et al.[49]
FA	GOH group; POH group; POH/WSL group	Saliva; Supragingival plaque	3 months	The counts of the CFUs of *Candida species*, *Streptococcus mutans*, and *Lactobacilli*	DMFT	Klaus et al.[56]
FA	FA group	Saliva	T0 (baseline), T1 (6 weeks), T2 (12 weeks), T3 (18 weeks)	*Candida albicans*, *Streptococcus mutans,* and *Lactobacillus acidophilus* counts	Salivary flow rate and pH	Arab et al.[57]
FA	FA group; Control group	Saliva	T0 (baseline), T1 (6 months)	Dentocult SM strips and the Dentocult LB method	Salivary pH and buffer capacity	Maret et al.[58]
FA	WSL group; Control group	Saliva	T0 (baseline), T1 (12 months)	16s rDNA sequencing	Modified WSL index, salivary Stephan curve kinetics, pH	Catunda et al.[59]
FA	CB group; SLB group	Saliva	T1 (baseline), T2 (3 months), T3 (6 months), T4 (18 months)	16S rRNA sequencing; qPCR	salivary sIgA, MPO, and LDH	Jing et al.[60]
FA	FA group	Saliva; Supragingival plaque	T0 (baseline), T1 (1 month)	metagenomic sequencing	GI, PLI, PD	Liu et al.[61]
FA	High altitude group; Sea level group	Saliva	T0 (baseline), T1 (>3 months)	16S rRNA sequencing	–	AlShahrani et al.[62]
FA	FA group	Saliva	T0 (baseline), T1 (6 months), T2 (appliance removal)	16S rRNA sequencing	–	Kado et al.[63]
FA	FA group; Control group	Saliva	10-12 months	PCR-DGGE; qPCR	–	Sun et al.[64]
FA	FA group	Saliva; Supragingival plaque	T0 (baseline), T1 (1 week), T2 (6 weeks), T3 (12 weeks)	RNA sequencing	BOP, GI, PD, PLI	Babikow et al.[48]
FA	FA group	Saliva	T0 (baseline), T1 (6 weeks), T2 (12 weeks), T3 (18 weeks)	total bacteria count; *Streptococcus* and *Lactobacillus* counts	MGI, GBI, PLI, IL-1β, MIF	Chen et al.[65]

the shift in oral microbiome from healthy to periodontitis.[63] Another experiment was conducted to investigate the salivary microbial diversity among FA orthodontic patients and healthy individuals. Saliva samples were collected and analyzed by Polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) during the midterm of orthodontic treatment (10–12 months). The experiment discovered a greater number of *Pseudomonas* spp. in the orthodontic group, which might be detrimental to a healthy oral environment.[64]

Besides, currently, saliva as a diagnostic tool is of public interest and some salivary microorganisms can be considered as oral disease discriminatory biomarkers.[31,70] In 2023, Babikow et al.[48] discovered that the relative abundance of *Stomatobaculum longum* and *Mogibacterium diversum* in prebonding saliva samples

was predictive of higher bleeding on probing (BOP) in 12 weeks after orthodontic appliance bonding, whereas *Neisseria subflava* was associated with lower BOP. Very few studies have investigated the relationships between pro-inflammatory cytokines and microbiological creatures among orthodontic patients (Figure 3A). In 2021, Chen et al.[65] investigated the associations between salivary inflammatory mediators and periodontal and microbiological creatures in orthodontic patients. In this study, positive correlations were found between salivary Interleukin-1Beta (IL-1β) and macrophage migration inhibitory factor (MIF) levels and total salivary bacteria count, and similar correlation coefficients between the total bacteria count (aerobic and anaerobic), *Streptococci* count, and *Lactobacilli* count with IL-1β levels which suggested that there were no specific bacteria combinations that might be associated with gingivitis. IL-1β and MIF may be useful and suitable biomarkers reflecting bacterial loads in the oral cavity.

Effect of fixed appliances on dental plaque biofilms

Dental caries and periodontitis have been characterized as bacterial infectious diseases with dental plaque as the initiating factor, encompassing supragingival plaque and subgingival plaque. Supragingival plaque develops in an environment directly exposed to the oral cavity, which is influenced by mastication as well as by salivary flushing and host defense components, which limit the accumulation of bacteria. In addition to the strong association between supragingival plaque and dental caries, it has also been demonstrated that there exists a certain relationship between supragingival plaque and the development of periodontitis.[71,72] Subgingival plaque is located in a closed environment, lacks salivary flushing and self-cleaning effect, is less susceptible to salivary defense components, is more protective than the setting of supragingival plaque, and is more intimately associated with periodontal disease-related bacteria..[39,71–73] The present review summarized a decade of research on the effects of FAs on the microbiome of supragingival and subgingival plaque (Table 2).

Effect of fixed appliances on the supragingival plaque microbiome

Supragingival plaques in patients wearing FAs showed different microbial compositions, which may be involved in some orthodontic complications, such as WSLs and dental caries. Klaus et al.[56] also investigated the effects of FAs on the supragingival plaque microbiome of the GOH group, POH group, and POH/WSL group after 3 months of orthodontic treatment. *S. mutans* was found in the plaque samples of all patients and *Lactobacilli* were found in 90.7% of the plaque samples, which represented the transition and shift of the supragingival plaque to cariogenic properties in orthodontic patients with FAs. Employing the RNA sequencing method, Babikow et al.[48] obtained similar conclusions. Among the 32 literature-identified periodontal and cariogenic pathogens selected, 8 species (*Streptococcus sanguinis, Eubacterium nodatum, Lachnoanaerobaculum saburreum, Selenomonas sputigena, Granulicatella elegans, Campylobacter gracilis, Corynebacterium matruchotii, and Leptotrichia wadei*) underwent significant changes in supragingival plaque over time. The abundance of *Lachnoanaerobaculum saburreum*, *Selenomonas sputigena*, *Campylobacter gracilis*, *Corynebacterium matruchotii*, and *Leptotrichia wadei* displayed a steady increase while the abundance of *Streptococcus sanguinis* showed a significant, lasting decrease from T0 (baseline) to T3 (12 weeks after FA treatment). *Streptococcus sanguinis* was recognized as a health-associated species that could antagonize cariogenic pathogens.[93]

An experiment explored the differences between orthodontic patients with CAs and patients with FAs for more than 6 months. Supragingival plaques of each patient were collected from both buccal and lingual sides, which were divided into four groups: plaques on the buccal side in patients with FAs (FB), plaques on the lingual side in patients with FAs (FL), plaques on the buccal side in patients with CAs (IB), plaques on the lingual side in patients with CAs (IL). It is demonstrated that some key communities were significantly enriched in the FB group, including *Coprobacillus*, *Bifidobacterium*, *Enterobacterium*, *Lactobacillus,* and so on, which might adversely affect the tooth hard tissue structure.[74] Additionally, Yang et al.[50] elucidated the distinguished supragingival plaque microbiota between patients with and without WSLs, who were undergoing FA orthodontic treatment within 6–12 months. *Candida albicans* was found to be frequently present and enriched in orthodontic-derived WSLs, which indicated that *Candida albicans* could shape supragingival plaque bacteria microbiome in demineralized lesions and might play a key role in WSL pathogenesis (Figure 3C). Through 16S rRNA gene sequencing, another research assessed the effect of 2 different frequently employed treatment types, FAs and CAs, on the supragingival plaque microbiome. Researchers conducted a long-term follow-up of 12 months. According to the results, elevated plaque indexes (PI) and gingival indexes (GI) in the FA group are associated with a higher abundance of disease-related genera. For example, the relative abundance of *Selonomonas* and *Leptotrichia* elevated with higher PI, a linear relationship was apparent only in the patients with FA. *Selonomonas*, *Leptotrichia*, *Veillonella*, *Prevotella*, and *Saccharibacteria* displayed an elevated level with higher GI, whereas the relative abundance of *Capnocytophaga*, *Haemophilus*, *Rothia*, *Cardiobacterium*, and *Kingella* decreased with increasing GI, which was also only observed for patients with FAs.[75]

What is more, it has been demonstrated that there is a certain relationship between alterations in supragingival plaque caused by FAs and orthodontic complications of periodontal disease. Recently, Liu et al.[60] demonstrated that *Rothia dentocariosa* and *Cardiobacterium hominis* are oral periodontal disease pathogens significantly increased in the supragingival plaques of patients wearing FAs for 1 month. For microbial metabolic pathway enrichment analysis, anaerobic energy metabolism invertebrates cytosol and so on were enriched in patients after orthodontics for 1 month, whereas heme biosynthesis and so on were enriched in patients before orthodontics, which means that changes in oral microbiome caused by orthodontic treatment were detrimental to periodontal health. Similarly, it was also confirmed that the levels of periodontal disease-associated bacteria increased during orthodontic treatment employing FAs.[76] A novel investigation was conducted to identify microorganisms isolated from patients with FAs for 3–6 months and to assess their resistance to different antimicrobials. The findings suggested that there was a more complicated supragingival

Table 2. A summary of the researches on the effect of fixed appliances on oral microbiome in dental plaque and other habitats

Appliances	Groups	Collection samples	Time points	Microbial detection techniques	Related parameters	Reference
FA	GOH group; POH group; POH/WSL group	Saliva; Supragingival plaque	3 months	The counts of the CFUs of *Candida species*, *Streptococcus mutans*, and *Lactobacilli*	DMFT	Klaus et al.[56]
FA	FA group	Saliva; Supragingival plaque	T0 (baseline), T1 (1 month)	metagenomic sequencing	GI, PLI, PD	Jing et al.[60]
FA	FA group	Saliva; Supragingival plaque	T0 (baseline), T1 (1 week), T2 (6 weeks), T3 (12 weeks)	RNA sequencing	BOP, GI, PD, PLI	Babikow et al.[48]
FA; CA	FA group (buccal); FA group (lingual); CA group (buccal); CA group (lingual);	Supragingival plaque (buccal and lingual sides)	>6 months	16s rDNA sequencing	–	Xie et al.[74]
FA	WSL group; Control group	Supragingival plaque	6-12 months	16S rRNA sequencing	–	Yang et al.[50]
FA; CA	FA group; CA group;	Supragingival plaque; Plaque from CA tray	T0 (baseline), T1 (1 month), T2 (3 months), T3 (6 months), T4 (12 months)	16S rRNA sequencing	GI, PLI	Shokeen et al.[75]
FA	FA group	GCF; Supragingival plaque	T0 (baseline), T1 (during the treatment)	Mass spectroscopy; PCR	DMFT, GI, PLI	Marincak Vrankova et al.[76]
FA; CA	FA group; CA group	Supragingival plaque	3-6 months	AST; VITEK 2	–	Pellissari et al.[77]
FA	band group; bonded tube group	Supragingival plaque	T0 (intervals during treatment), T1 (up to 1 year after appliance removal)	Denaturing gradient gel electrophoresis and 16S rDNA microarray	–	Ireland et al.[78]
FA	band group; bonded tube group	Subgingival plaque	T0 (baseline), T1 (4–7 weeks)	DNA-strip tehnique	–	Mártha et al.[79]
FA	FA group; Control group	Subgingival plaque	T0 (baseline), T1 (6 weeks), T2 (12 weeks)	16S rRNA sequencing	PD, GR, CAL, BOP	Chen et al.[80]
FA	FA group	Subgingival plaque	T0 (baseline), T1 (1 month), T2 (3 months)	16S rRNA sequencing	PLI, GBI	Guo et al.[81]

(*Continued on next page*)

Table 2. *Continued*

Appliances	Groups	Collection samples	Time points	Microbial detection techniques	Related parameters	Reference
FA; CA	FA group; CA group	Subgingival plaque	T0 (baseline), T1 (3 months), T2 (6 months)	Phase-contrast microscope analysis	–	Caccianiga et al.[82]
FA	FA group	Subgingival plaque	T0 (baseline), T1 (12 months)	Checkerboard DNA-DNA hybridization	PLI, PD, CAL	Lemos et al.[83]
FA	FA group; Control group	Subgingival plaque	T0 (baseline), T1 (1 month), T2 (2 months), T3 (3 months), T4 (6 months)	PCR	GI, *fimA* genotypes of *Porphyromonas gingivalis*	Pan et al.[84]
FA	FA group; Control group	Subgingival plaque	T0 (baseline), T1 (10 days after debonding)	PCR	GBI, PLI	Yáñez-Vico et al.[85]
FA	FA group; Control group	Subgingival plaque	T0 (debonding), T1 (1 month after debonding), T2 (3 months after debonding)	Real-time PCR	GI, PD, SBI	Pan et al.[86]
FA	Placement group; Removal group	Subgingival plaque	T0 (baseline), T1 (1 month after placement/removal), T1 (3 months after placement/removal)	PCR	–	Sandić et al.[87]
FA	SLB group; CB group	GCF	T0 (baseline), T1 (30 days), T2 (60 days)	CTAB-DNA precipitation method; Real-time PCR	–	Bergamo et al.[88]
FA	Control group; The second group (pregnant women who had previously used FAs); The third group (pregnant women with current FAs)	Mucosal swabs	–	qPCR	OHI-S	Kurniawan et al.[89]

(*Continued on next page*)

Table 2. *Continued*

Appliances	Groups	Collection samples	Time points	Microbial detection techniques	Related parameters	Reference
FA	Metal bracket group; Ceramic bracket group	Mucosal swabs	T0 (baseline), T1 (1 month), T2 (6 months), T3 (1 year), T4 (6 months after debonding)	*Candida albicans* counts	–	Sanz-Orrio-Soler et al.[90]
FA	FA group	Mucous membrane of dorsal tongue swabs	–	–	–	Gavrilova et al.[91]
FA	FA group	Oral rinses and elastomeric ligature samples	T0 (baseline), T1 (2 weeks), T2 (6 weeks), T3 (12 weeks)	SEM; *Candida albicans* counts	API, GBI	Grzegocka et al.[92]

FA: fixed appliances; GOH: good oral hygiene; POH: poor oral hygiene; POH/WSL: poor oral hygiene with white spot lesions; CFU: colony forming unit; DMFT: decayed, missing and filled teeth index; GI: gingival index; PLI: plaque index; PD: probing depth; BOP: bleeding on probing; CA: clear aligner; GCF: gingival crevicular fluid; CB: conventional bracket; SLB: self-ligating bracket; PCR: polymerase chain reaction; AST/VITEK 2: microbial identification and antimicrobial susceptibility testing; GR: gingival recession; CAL: clinical attachment level; SBI: sulcus bleeding index; qPCR: quantitative real-time PCR; OHI-S: simplified oral hygiene index; SEM: scanning electron microscopy; API: approximal plaque index.

plaque biofilm with a higher level of bacterial resistance in patients wearing FAs and 14 of 19 isolated strains were observed to be resistant to at least one of the antimicrobials tested.[77] In addition, Ireland et al.[78] conducted an experiment dedicated to examining longer-term microbial alterations of supragingival plaques in patients undergoing orthodontic treatment with FAs. The findings suggested that progressive changes in supragingival plaque composition and structure occur during treatment, with no indication of restoration to the pretreatment microbiome, even 1 year after removal.

Effect of fixed appliances on the subgingival plaque microbiome

FAs could lead to changes in the microbial composition, structure, and metabolic function of the subgingival plaque biofilm, which increased the level of periodontopathogens in subgingival plaque, and most investigations have revealed that these detrimental periodontal changes were reversible after the removal of FAs.

An experiment was designed to evaluate the prevalence of 11 periodontopathogens in subgingival plaque biofilm of banded molars and bonded tube molars in patients after 4–7 weeks of bonding the FAs and a slight increase was observed in both groups.[79] Additionally, Chen et al.[80] provided the first longitudinal, culture-free, and deep-sequence profiling of subgingival plaque microbiome in patients during the early stages of orthodontic treatment. The study confirmed that FA insertion elicited increased microbial richness, accompanied by the increased incidence of localized gingivitis/mild periodontitis compared to the control group. Meanwhile, it was confirmed that individual- and site-dependent microbiome variability existed in subgingival plaque. For example, molar bands might lead to more prolonged shifts in subgingival plaque compared to orthodontic brackets. Similarly, another study also investigated the subgingival microbial alterations during the first 3 months in female adult patients wearing FAs. At the species level, *Prevotella intermedia*, *Campylobacer rectus*, *Fusobacterium nucleatum*, and *Treponema denticola* elevated without significant differences, which might indicate that the subgingival plaque microbiome affected by FAs could contribute to the transient mild gingival inflammation.[81] The unfavorable microbiota in subgingival plaque of patients wearing FAs and CAs was analyzed by phase-contrast microscope. The outcomes revealed that the risk of developing unfavorable microbiota increases in the FA group compared CA group.[82] Lemos et al.[83] conducted a long-term investigation aimed at assessing the impact of FA insertion on the subgingival microbiota and periodontium in adult patients. Subgingival biofilm samples were collected at baseline and after 12 months of treatment and were analyzed by checkerboard DNA-DNA hybridization. A significant decrease in the proportions of the *Actinomyces species* and an increase in the orange complex species were observed. However, the abundance of the red complex species was observed to be unchanged. What is more, another research explored the correlation between the prevalence of *fimA* genotypes of *Porphyromonas gingivalis* and periodontal status in adolescent patients with FAs, and *Porphyromonas gingivalis fimA* II or IV was confirmed to be closely associated to orthodontic gingivitis.[84]

In addition, several studies have explored alterations in subgingival plaque microorganisms after the removal of orthodontic FA appliances. Ireland et al.[78] found that compared to bonded molar tubes, the molar bands were more likely to elicit changes in the supragingival plaque microbiome, enabling a shift toward a microbiome with higher periodontal disease potential. A study was carried out to evaluate the short-term effect of removal of FAs on subgingival microbiota and compare the microbiological and clinical parameters in patients with FAs and patients 10 days after the bracket removal. The prevalence of *Treponema denticola* had a significant decrease 10 days after bracket removal and a significant positive correlation was observed between BOP and *Aggregatibacter actinomycetemcomitans* and between clinical parameters and *Prevotella intermedia* at 10 days after bracket removal. The findings suggested that FA insertion influenced the subgingival plaque microbiome and contributed to more inflammation and bleeding while the effect of orthodontic appliance removal on subgingival microorganisms facilitated periodontal health status.[85] Pan et al. collected subgingival plaque samples from the lower incisors in patients wearing FAs at baseline (T0), 1 month after orthodontic treatment (T1) and 3 months after orthodontic treatment (T2). At T2, the prevalence of periodontal pathogens tended to return to normal, while the amount of *Prevotella intermedia* remained high. The subgingival microbiome changes caused by FAs were only partially reversible at 3 months after orthodontic appliance removal.[86] However, some researchers conducted a 3-month experiment and demonstrated that in the first months after the insertion and removal of the fixed orthodontic appliances changes in the subgingival microbiome were not significant, which could be attributed to good oral hygiene.[87]

Effect of fixed appliances on the oral microbiome in other habitats

An experiment was designed to collect the gingival crevicular fluid (GCF) and dental plaque from 30 orthodontic patients before bonding of FAs (T0) and during the treatment (T1), which were analyzed for selected *Candida species* and for 10 selected oral bacteria employing mass spectroscopy and PCR, respectively. Unfavorable changes in oral microorganisms and oral health status were recorded in the medium term after bonding of FAs.[76] In addition, another study evaluated the influence of self-ligating brackets and conventional brackets with elastomeric ligatures on the GCF levels of the putative periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans sorotype a*, *Tannerella forsythia*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*. The GCF samples were collected at baseline (T0) and after 30 (T1) and 60 (T2) days after bonding of brackets. In this study, we could observe that the levels of bacterial species associated with periodontal disease *Porphyromonas gingivalis* significantly increased in the self-ligating brackets. Good oral hygiene maintenance strategies should be adopted in the use of the self-ligating brackets.[88]

Kurniawan et al.[89] investigated the oral cavity microbiome profiles in mucosal swabs, inflammatory factor IL-6, and TNF-α in the saliva of pregnant women using FAs. Compared to the pregnant women who had never used orthodontic appliances (control group), the pregnant women who had previously used orthodontic appliances had no significantly different levels of seven types of bacteria from the genera *Streptococcus*, *Gemella*, *Lactobacillus*, and *Abiotrophia*. No significant differences were observed in the IL-6 levels in the pregnant women with current orthodontic appliances, while the level of TNF-α in the pregnant women with current orthodontic appliances was higher compared with the control group. Therefore, the application of FAs during pregnancy should be allowed under certain conditions. Another research quantified the colonization of *Candida albicans* in patients before orthodontic treatment and during orthodontic therapy. Oral mucosal swabs were collected and the colonies were calculated by Sabouraud Dextrose Agar plates. The experiment suggested that FAs had no influence on the presence, absence, or level of colonization by *Candida albicans* and there were no significant differences between the different appliances studied.[90] What is more, Gavrilova et al.[91] analyzed the spectrum, rate of occurrence, and amounts of oral microorganisms on the mucous membrane of the dorsal tongue before orthodontic treatment and during different treatment stages. The amounts of opportunistic pathogenic microorganisms varied during different treatment stages.

What is more, 17 patients wearing conventional brackets were recruited, and oral rinses and elastomeric ligature samples were collected in order to assess *Candida* prevalence in the oral microbiome. The study revealed that the amount of *Candida* in the mouth showed some fluctuation during the treatment but in general had an upward trend.[92]

In conclusion, the introduction of FAs could lead to alterations in the composition and structure of oral microbiomes in different habitats including saliva, supragingival plaque, subgingival plaque, and others), which could increase the risk of oral and even systemic diseases in patients undergoing orthodontic treatment (Table 2).

ALTERATIONS OF THE ORAL MICROBIOME IN ORTHODONTIC PATIENTS WITH CLEAR ALIGNERS

In contrast to FAs, CAs have no bracket and steel wire and are favored by the majority of orthodontic patients because of their aesthetics, clearness, and comfort.[94–96] From a macro perspective, most previous studies concluded that CAs are more conducive to demonstrating a higher level of oral health,[97–99] including dental[98] and periodontal health.[99] In line with the above, currently some scholars agree that CA orthodontic treatment could not exert deleterious effects on the oral microbiome. And if oral hygiene was properly promoted, CAs did not lead to significant changes in the biodiversity of the oral microbial community. However, there are still some studies showing that CAs can trigger significant changes in the oral microbiota (Figure 4).

Effect of clear aligners on the salivary microbiome

Numerous studies have indicated that CAs do not elicit the deterioration of oral health nor significant biodiversity changes in salivary microbiota after detailed oral hygiene instructions have been provided (Table 3).

Mummolo et al. compared the saliva levels of *S. mutans and Lactobacilli* in patients wearing CAs and multibracket orthodontic appliances. They enrolled 80 subjects and collected saliva

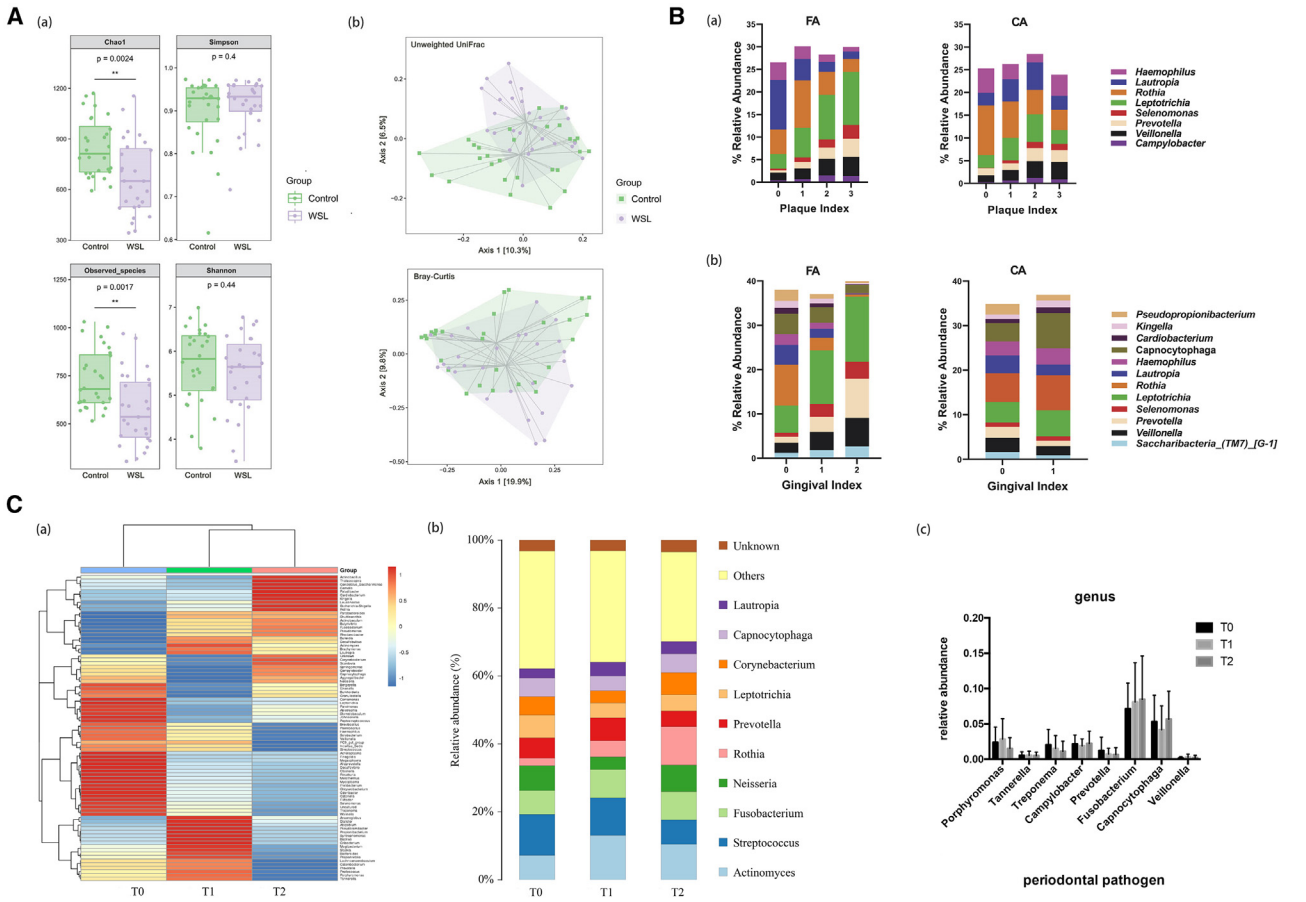

Figure 4. Schematic illustrations of alterations of the oral microbiome in orthodontic patients with clear aligners (CAs)

(A) (a) Boxplots of alpha diversity of salivary microbiota in the control and white spot lesion groups. Left: Chao1 and Observed_species indices; right: Shannon and Simpson indices (**$p < 0.01$, Kruskal-Wallis). (b) PCoA plot based on the unweighted UniFrac and Bray-Curtis distances. A certain trend of clustering and separation of samples was identified when comparing the control and white spot lesion groups ($p < 0.05$, PERMANOVA).

(B) Percentage relative abundance of genera in supragingival plaque exhibiting differences in association with (a) plaque index in FAs (left) and CAs (right) and with (b) gingival index in FAs (left) and CAs (right).

(C) Analysis of the change of subgingival microbial structure at the genus level in female adult patients with clear aligners. (a) Heatmap of relative abundance of subgingival bacteria at genus level at three different time points: before orthodontic treatment (T0), one month after orthodontic treatment (T1), and three months after orthodontic treatment (T2). (b) Genus-level taxon distribution at T0, T1, and T2. (c) The relative abundance of eight periodontal pathogens at the genus level within three different time points. Adapted with permission from.[75,100,101] Copyright 2024, Elsevier.

samples from patients prior to the start of orthodontic treatment (T0), and after 3 months (T1) and 6 months (T2). The results suggested that compared with that of the multibracket orthodontic appliance group, the CA-treated subjects achieved lower saliva levels of *S. mutans* and *Lactobacilli* after 6 months of treatment.[102] Similarly, in another study, saliva and periodontal parameters were sampled from patients wearing CAs or FAs before treatment (T0), and after 3- (T2) and 6-month (T3) orthodontic treatments. The outcomes revealed that the relative abundances of 3 bacterial genera and 15 species significantly increased in the FA group whereas they remained stable in the CA group. And among them, bacterial genera *Selenomonas*, *Stomatobaculum*, *Olsenella,* and *Faecalicoccus* and bacterial species *Selenomonas_sputigena*, *Dialister_invisus*, *Olsenella_profus*, *Prevotella_buccae*, *Cryptobacterium_curtum,* and *Clostridium_spiroforme* were significantly positively correlated with periodontal parameters.[103] Zhao et al.[104] evaluated the effects of Invisalign aligners on patients' oral bacterial communities and oral health. Their experiment involved 25 adult subjects who were treated with Invisalign appliances and their saliva samples were collected before, and six months after orthodontic treatment. Through 16S rRNA gene sequencing, a total of 1,853,952 valid reads were obtained from 50 saliva samples from the subjects, with an average of 37,904 sequences per sample. There were no significant differences in biodiversity between and within the two groups. A total of 8885 operational taxonomic units (OTUs) were identified by clustering and classified into six major phyla: Firmicutes, Proteobacteria, Bacteroidetes, Clostridium, actinobacteria, and Candidate_division_TM7_norank. At the genus level, there was a significant increase in the abundance of *Bacillus* and a significant decrease in the abundance of *Prevotella* in Invisalign patients after 6 months compared with those before

Table 3. A summary of the researches on the effect of clear aligners on oral microbiome in saliva

Appliances	Groups	Collection samples	Time points	Microbial detection techniques	Related parameters	Reference
FA CA	FA group; CA group	Saliva	T0 (baseline), T1 (3 months), T2 (6 months)	*Streptococcus mutans* and *Lactobacilli* counts	PLI, salivary flow, buffering power of saliva	Mummolo et al.[102]
FA CA	FA group; CA group	Saliva	T0 (baseline), T1 (3 months), T2 (6 months)	16S rRNA sequencing	PLI, GI, PD	Wang et al.[103]
CA	CA group	Saliva	T0 (baseline), T1 (6 months)	16S rRNA sequencing	BOP, PLI, PD	Zhao et al.[104]
FA CA	FA group; CA group	Saliva	6 months	16S rRNA sequencing	–	Wang et al.[105]
CA	WSL group; Health group	Saliva	>1 year	16S rRNA sequencing; UPLC-MS/MS	–	Song et al.[100]

FA: fixed appliances; CA: clear aligners; PLI: plque index; GI: gingival index; PD: probing depth; BOP: bleeding on probing; WSL: white spot lesions; UPLC-MS/MS: ultra-performance liquid chromatography-tandem mass spectrometry.

treatment, which might suggest a healthier oral status and less risk of periodontitis.

However, another study came to a different conclusion: compared with FAs, the effect of the Invisalign appliances on the oral saliva microbiome was no better for oral health.[105] Research suggested that in the Invisalign group, the abundance of Firmicutes at the phyla level was less than that in the FA group and the abundance of TM7 was significantly higher. At the genus level, there was a higher level of *Neisseria* in the Invisalign group compared with that in the FA group.[105] In addition, Song et al.[100] collected the saliva samples of adolescents with WSLs (n = 81) and those without WSLs (n = 124), who had been treated with CAs for more than one year. The saliva samples were analyzed by 16S rRNA sequencing and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and the analysis revealed distinct taxa in the salivary microbiome of patients with WSLs and those without WSLs. There were 14 taxa, including *Actinobacteria*, *Actinomycetales*, *Rothia*, *Micrococcaceae*, *Subdoligranulum*, *Capnocytophaga*, *Azospira*, *Olsenella*, *Lachnoanaerobaculum*, and *Abiotrophia*, with higher relative abundances in the WSL group. Moreover, the findings suggested that a concordant increase in the levels of *Lachnoanaerobaculum*, *Rothia*, *Subdoligranulum,* and some amino acids had predictive value for WSL development, which might provide candidate salivary biomarkers for the diagnosis and treatment of WSL associated with CAs. In summary, when teenagers received CA treatment with poor oral hygiene practices over a long period of time, the CAs could disrupt the balance of the oral micro-ecosystem and increase the risk of developing oral diseases (Figure 4A).

Effect of clear aligners on the microbiome in dental plaque biofilms

With well-maintained oral hygiene, the vast majority of studies have shown that CAs have a more negligible impact on dental plaque microorganisms compared to FAs, meaning that CAs are beneficial for oral health and more suitable for patients at risk of periodontal disease (Table 4).

A longitudinal study compared the supragingival plaque microbiota changes during orthodontic treatment using FAs and CAs. The microbiome composition of these supragingival plaques was determined using 16S rRNA gene sequencing. The results revealed that beta diversities of the supragingival microbial communities were distinct between FA groups and CA groups and increased periodontal parameters PI and GI in the FA group correlated with a higher abundance of disease-associated genera, which indicated CAs could cause a better oral health status compared to FAs (Figure 4B).[75] However, the experiment by Xie et al.[74] yielded inconsistent results. They explored the differences in supragingival plaque microbial communities between the CA group and the FA group for more than 6 months. Supragingival plaque was collected from both buccal and lingual sides, which were divided into four groups: FB group, FL group, IB group, and IL group. It is demonstrated that the IB group showed higher relative abundances of *Actinomycetes* and *Rosella*, which were considered to be associated with caries and periodontal disease and bad for oral health.

In addition, studies on the influence of CAs on subgingival plaque community profiles showed favorable results.[82,101,106] Levrini et al.[106] compared the subgingival microbiota changes using real-time PCR in patients treated with FAs and an Invisalign aligner for 3 months. Compared with that of the FA group, the Invisalign group revealed better results in terms of total biofilm mass. In addition, the Invisalign group was free of periodontal pathogens, while in the fixed orthodontic appliance group, a periodontopathic bacteria was detected in one patient. Another study investigated the changes in subgingival plaque microbiota in 10 female patients who were undergoing transparent CA treatment. Researchers collected subgingival plaque samples at three time points: before orthodontic treatment (T0), 1 month after orthodontic treatment (T1), and 3 months after orthodontic treatment (T2). The study revealed that there was a slight decrease in the microbial diversity and a significant change in the microbial structure during 3-month of treatment with CAs, although the levels of periodontal microorganisms and core microorganisms were relatively stable (Figure 4C).[101] Rouzi et al.[107]

Table 4. A summary of the researches on the effect of fixed appliances on oral microbiome in dental plaque and other habitats

Appliances	Groups	Collection samples	Time points	Microbial detection techniques	Related parameters	Reference
FA CA	FA group; CA group	Aligner tray plaque; Supragingival plaque	T0 (baseline), T1 (1 month), T2 (3 months), T3 (6 months), T4 (12 months)	16S rRNA sequencing	PLI, GI	Shokeen et al.[75]
FA; CA	FA group (buccal); FA group (lingual); CA group (buccal); CA group (lingual);	Supragingival plaque (buccal and lingual sides)	>6 months	16s rDNA sequencing	–	Xie et al.[74]
FA; CA	FA group; CA group; Control group	Subgingival plaque	T0 (baseline), T1 (1 month), T2 (3 months)	Real-time PCR	PLI, PD, BOP	Levrini et al.[106]
CA	CA group	Subgingival plaque	T0 (baseline), T1 (1 month), T2 (3 months)	16S rRNA sequencing	PLI, GBI	Guo et al.[101]
CA	CA group	Aligner tray plaque; Subgingival plaque	T0 (baseline), T1 (1 month), T2 (3 months)	16S rRNA sequencing	PLI, PD, BI	Rouzi et al.[107]
FA; CA	FA group; CA group	Subgingival plaque	T0 (baseline), T1 (3 months), T2 (6 months)	Phase-contrast microscope analysis	–	Caccianiga et al.[82]
CA	CA group	Saliva; Aligner tray plaque	T0 (baseline), T1 (4 h), T2 (8 h), T3 (12 h), T4 (24 h)	16S rRNA sequencing	–	Yan et al.[108]

FA: fixed appliances; CA: clear aligners; PLI: plque index; GI: gingival index; PD: probing depth; PCR: polymerase chain reaction; BOP: bleeding on probing; GBI: gingival bleeding index; BI: bleeding index.

conducted research collecting the subgingival plaque sample of patients with CA at three time points: before the initiation of aligner treatment (T0), 1 month after treatment onset (T1), and 3 months after treatment onset (T3). The results showed that CA treatment had no significant impact on the subgingival microbiota composition. In addition, Caccianiga et al.[82] applied phase-contrast microscopy to the microbiological analysis of subgingival plaque in 50 patients (25 patients fitted with FAs and 25 patients fitted with CAs for 6 months). The results revealed that the risk of developing pathogenic bacterial flora in patients treated with multibracket appliances was higher than in those treated with CAs.

Effect of clear aligners on the microbiome in the plaque from the aligner tray

Researchers have explored the influence of CAs on the plaque from the aligner tray. Yan et al.[108] collected saliva samples and the contents of the inner surface of the aligner tray at 0 h (T0), 4 h (T4), 8 h (T8), 12 h (T12), and 24 h (T24) after aligner placement. Applying 16S rRNA gene sequencing, the results showed a decrease in alpha diversity values and the abundance of specific microbes on the inner surface of the aligner tray from T0 to T24, while there was an insignificance increase in the beta diversity of the microbial composition from T0 to T24. In addition, compared with those at T0, the relative abundances of phylum Firmicutes, orders Lactobacillales, and Bacteroidales, and the genus *Streptococcus* and species *Streptococcus infantis* increased significantly, while those of genera *Actinomyces* and *Rothia*, and the species *Rothia aeria* decreased significantly at T24, which suggested that uncleaned CAs might cause enamel damage. Another study revealed that there was a unique plaque community in the inner surface of the aligner tray using 16S rRNA gene sequencing.[75] Similarly, Rouzi et al. collected the plaque samples from the inner surface of aligners before treatment, 1 month after treatment, and 3 months after treatment and the findings displayed that the relative abundance of *Streptococcus* increased significantly, as well as the richness and diversity of microbiome decreased substantially with increasing treatment time.[107]

ALTERATIONS OF THE ORAL MICROBIOME IN ORTHODONTIC PATIENTS WITH OTHER APPLIANCES

In addition to fixed and clear appliances, other types of appliances are also applied in the orthodontic treatment process and exert an effect on the oral microbiome (Table 5).

Various types of removable orthodontic appliances have also been demonstrated to adversely affect the oral microbiome. A study investigated the salivary concentrations of *S. mutans* and some *Lactobacilli* in patients with removable positioners.

Table 5. A summary of the researches on the effect of other orthodontic appliances on oral microbiome

Appliances	Groups	Collection samples	Time points	Microbial detection techniques	Related parameters	Reference
FA; CA; RP	FA group; CA group; RP group	Subgingival plaque	T0 (baseline), T1 (3 months), T2 (6 months)	*Streptococcus* and *Lactobacillus* counts	PLI	Mummolo et al.[109]
Clear orthodontic retainer	–	Saliva; Plaque from clear retainer	T0 (1 day), T1 (7 days), T2 (14 days)	16S rRNA sequencing	–	Velliyagounder et al.[110]
Thermoplastic retainers	Patient group; Health group	Saliva; Swabs from tooth surface	<3 months	the isolation and identification of the bacteria	pH, IgA levels	Al-Lehaibi et al.[111]
FR; RR	FR group; RR group; Lower FR and upper RR group	Salivary swabs	T0 (the first immediately after debonding), T1 (6 weeks after debonding),	RT-PCR	PLI, GI, PD, BOP, WSL, DMFT	Lucchese et al.[112]
Expander	RPE group; Mc Namara group; Control group	Saliva	T0 (baseline), T1 (3 months), T2 (6 months)	*Streptococcus* and *Lactobacillus* counts	–	Ortu et al.[113]
TADs	The failed group; The successful group	TADs	–	16S rRNA sequencing; Metagenomic sequencing; qRT-PCR	–	Zhao et al.[114]
TADs	Health group; Periodontitis group	TADs	–	16S rRNA sequencing; SEM	–	Zhao et al.[115]

FA: fixed appliances; CA: clear aligners; RP: removable positioner; PLI: plaque index; IgA: Immunoglobulin A; FR: fixed retention devices; RR: removable retention devices; qRT-PCR: quantitive real-time PCR analysis; GI: gingival index; PD: probing depth; BOP: bleeding on probing; WSL: white spot lesion; DMFT: decayed, missing and filled teeth index; RPE: rapid palatal expander; TADs: temporary anchorage devices; SEM: scanning electron microscopy.

The results showed that the removable positioners contributed to microbial colonization (*S. mutans and Lactobacilli*), which might lead to a high risk of caries development, while the FAs achieved a higher level of microbial colonization.[109] Several studies investigated the impacts of clear retainers on oral microbiome. Saliva and plaques in the clear orthodontic retainers were collected during 14 days of treatment and Illumina MiSeq sequencing analysis revealed that compared to that in the saliva, the *Firmicutes* in plaque were significantly increased after 7 and 14 days of retainer treatment, while the *Campylobacteriota* were significantly decreased. At the genus level, several microbiotas showed significant increases in relative abundance in the clear retainer during the 14-day period. The results showed that the application of clear orthodontic retainers might lead to enamel change and periodontal tissue destruction.[110] Similarly, Al-Lehaibi et al. found that thermoplastic retainers contributed to the unfavorable change in the oral cavity microbiota.[111] In an experiment, 30 patients were enrolled and divided into three groups (group I: patients treated with upper and lower fixed retention devices, group II: patients with upper and lower removable retention devices, and group III: patients with lower fixed and upper removable retention devices). Lucchese et al.[112] evaluated the changes in the microbiome of salivary swabs among three groups at T0 (the first immediately after debonding) and T1 (the other one 6 weeks later) and analyzed the levels of six bacterial species (*Streptococcus mutans*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, and *Fusobacterium nucleatum*), which were most correlated with the development of caries and periodontal disease. The outcomes demonstrated that the levels of the bacterial species investigated in saliva tended to decrease at T2 and the oral microbiome tended to recover after orthodontic appliance removal.

Additionally, other studies have displayed that various types of expanders and temporary anchorage devices (TADs) inserted into the mouth have an adverse effect on the oral microbiome. In addition, another study enrolled 30 subjects aged 6–9 years and classified them into the rapid palatal expander group, the McNamara expander group, and the control untreated group. They assessed the saliva microbial levels of *S. mutans* and *Lactobacilli* during rapid palatal expansion, which showed that the rapid palatal expander and the McNamara expander contributed to microbial colonization.[113] What is more, employing the 16S rRNA sequencing, metagenomic sequencing, and quantitative real-time PCR analysis (qRT-PCR) method, Zhao et al.[114] analyzed the differences in microbial composition and function between the failed temporary anchorage devices (TADs) and the successful TADs. The research found that in the failed group, *Prevotella intermedia*, *Eikenella corrodens*, *Parvimonas* spp, *Neisseria elongata*, and *Catonella morbi* were enriched. Zhao et al.[115] also explored the influence of the history of periodontitis on the microbiome colonization of the TAD surface. They used

scanning electron microscopy (SEM) to analyze whether there were biofilms on the TAD surface of the healthy group and the periodontitis group, and collected microorganisms on the TADs surface for analysis by 16S rRNA gene sequencing. The research discovered pathogens associated with periodontitis colonization on the TADs in the periodontitis group. At the species level, the components of the red complex, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* have discovered an increase in the periodontitis group.

IMPROVEMENT OF ORTHODONTIC APPLIANCES FROM A MICROBIOLOGICAL PERSPECTIVE

The introduction of orthodontic appliances promotes plaque retention and interferes with the oral microbiome, which subsequently adversely affects oral health and increases the potential risk of enamel demineralization, gingival disease, and periodontal disease.[47,58,105,116] Recently, there have been numerous researches dedicated to the improvement of the antimicrobial properties of orthodontic appliances, including brackets, archwires, adhesives, clear aligners, and so on.[94,117–120] These antimicrobial materials are categorized into four main categories: metals and metal compounds, inorganic non-metallic compounds, organic compounds, and bioactive materials. However, current antimicrobial strategies for orthodontic appliances are mainly devoted to the inhibition of cariogenic bacteria or the reduction of the total number of microorganisms, and fewer studies have been conducted on antimicrobial strategies for periodontal pathogens. These antimicrobial materials are divided into three main categories: metals and metal compounds, inorganic non-metallic compounds, and organic compounds. Among them, metals and metal compounds have been most widely applied in the improvement of antimicrobial properties of orthodontic appliances.

Metals and metal compounds

Metals and metal compounds have been applied most extensively in the improvement of antimicrobial properties of orthodontic appliances, mainly including silver and silver compounds,[121–130] titanium and titanium compounds,[131–137] zinc and zinc compounds,[138–144] and gold and gold compounds.[145–148] Similarly, the majority of studies applying metals and metal compounds to improve the antimicrobial properties of orthodontic appliances focused on their inhibitory effect on cariogenic bacteria, mainly S. mutans. Few studies have been conducted on the inhibition of periodontal pathogens.

Among the various metals, silver is well known for its antimicrobial activity against bacteria, fungi, protozoa, and certain viruses, and has shown good biocompatibility on numerous occasions.[149] Jasso-Ruiz et al.[123] assessed the antibacterial properties of surface-modified different orthodontic brackets with silver nanoparticles (AgNPs) against *S. mutans* and *S. sobrinus*. According to the results, the brackets with added AgNPs, the bacterial adherence of both microorganisms was lower than in brackets without the addition of AgNPs. Another research demonstrated that AgNPs could be added to commercial orthodontic adhesives to improve their bactericidal activity without altering their mechanical properties.[130] Nafarrate-Valdez et al.[129] found that AgNP could significantly inhibit bacterial growth against serotypes *c* and *k* of *S. mutans* strain on conventional orthodontic wires.

There is substantial evidence that titanium dioxide (TiO_2) nanoparticles can exhibit antimicrobial properties, and it is capable of generating hydroxyl radicals and reactive oxygen species under ultraviolet irradiation.[150,151] Thus, TiO_2 is broadly applied to improve the antimicrobial properties of orthodontic appliances. Ahmad Fauzi et al.[133] developed an aesthetic resin composite by applying a nitrogen-doped TiO_2 ($NTiO_2$) filler, which displayed an antimicrobial property against *S. mutans* under visible light exposure. Anand et al.[137] fabricated a functionalized bioactive orthodontic wire using TiO_2 NPs and Ag NPs, which have demonstrated an ability to inhibit the formation of oral biofilms and showed a strong antibacterial effect against multi-drug resistant bacteria in patients with a variety of oral diseases. Another study added TiO_2 NPs into the acrylic baseplates of twin block appliances, which significantly decreased the bacterial colony count after at least 4 months of application.[136] In addition, an experiment created a clear aligner that contained piezoelectric barium titanate nanoparticles (BaTiO3NPs), which have been demonstrated to have significant antimicrobial properties, resulting in a significant decrease in biofilm biomass.[131]

Zinc oxide (ZnO) exhibited excellent antibacterial properties, even at low concentrations and even under no light conditions. A study fabricated a novel orthodontic adhesive containing 5% Curcumin-doped ZnO NPs photoactivated with visible light. The novel adhesive displayed an enhanced antibacterial ability against *S. mutans*.[140] Another study examined the antimicrobial efficacy of ZnO NP-coated aligners and the results showed an excellent antimicrobial effect against *S. mutans* and a minimal antimicrobial effect against *Candida albicans*.[141]

The application of gold nanoparticles (AuNPs) can also enhance the antibacterial performance of CAs. An experiment successfully fabricated antibacterial 4,6-diamino-2-pyrimidinethiol-modified AuNPs, which were coated onto CAs. The results showed that the coated aligners had excellent antibacterial effects on a suspension of *Porphyromonas gingivalis*, and *in vivo* and *in vitro*, the newly developed nanomaterials showed excellent biocompatibility.[147]

Inorganic non-metallic compounds

Graphene oxide (GO) is one of the most promising nanomaterials in the field of dentistry due to its favorable biological properties, biocompatibility, high specific surface area, mechanical strength, and ease of synthesis process, as well as its low cost.[152–155] GO has a good antimicrobial property derived from the formation of reactive oxygen species, ‘‘nano-blade’’ and ‘‘wrapping’’ effect.[156] A study assessed the antimicrobial ability of orthodontic composite containing nano-structured GO (OC-nGO), which was recognized as a novel material following photodynamic therapy and photothermal therapy against *S. mutans* and could be applied as the orthodontic adhesive.[152] In another experiment, GO/Ag nanocomposite coatings were fabricated on the surface of orthodontic nickle-titanium (NiTi) alloy archwire, which exerted a stable antimicrobial effect against 90% of *S. mutans* for 7 days.[153]

Chlorhexidine (CHX) is a cationic bisbiguanide and can be attracted to bacterial cell walls with negatively charged and binds to inner membranes to increase cell wall permeability. CHX has broad-spectrum antimicrobial activity and is often employed as a mouthwash or rinse due to its water-soluble properties.[157] However, its application in orthodontics is limited.[157–160] Choi et al.[158] developed chlorhexidine-releasing elastomerics (CRE) and evaluated its anti-biofilm activity. The outcomes revealed that compared to 0.1% chlorhexidine mouthwash, CRE had better anti-biofilm and demineralization-inhibiting effects. According to another study, as a modifier of a commercial orthodontic adhesive, CHX-loaded poly-L-glycolic acid (PLGA) nanoparticles exhibited enhanced antimicrobial properties.[159] Fluoride also showed favorable antimicrobial and enamel remineralization performances. Yan et al.[161] developed a fluoride-coated clear aligner plastic (FCAP), which had excellent antimicrobial, fluoride recharge, and enamel remineralization performances.

Bioactive glasses (BAG) are a variety of bioceramic materials with excellent biocompatibility, and high antimicrobial features in the internal environment of the human body. Additionally, bioactive glasses possess excellent remineralization performance and are extensively applied in orthodontics.[162–167] Nam et al.[163] developed a novel orthodontic bonding resin containing fluorinated graphite and BAG (FGtBAG). The orthodontic bonding resin containing FGtBAG showed high antibacterial activity and a high concentration-dependent remineralization effect at 24 and 48 h. Choi et al.[166] added mesoporous bioactive glass nanoparticles to orthodontic self-adhesive resins in order to improve the physical properties and remineralization ability. The novel orthodontic self-adhesive resin showed a good antibacterial effect on both gram-negative and gram-positive bacteria.

In addition, Wang et al.[162] synthesized blue fluorescent carbon dots (HCDs) using the traditional Chinese medicinal honokiol, which exhibited good antibacterial effects on both gram-positive and gram-negative bacteria. Subsequently, they modified the surface of the orthodontic brackets with polydopamine and HCDs to enhance the antibacterial properties of the orthodontic brackets.

Organic compounds

A variety of organic compounds have been used to improve the antimicrobial properties of orthodontic appliances.[163–175] Chitosan is a copolymer of N-acetyl-D-glucose amine and D-glucose amine with excellent antibacterial, antifungal, and anti-inflammatory capacity, as well as excellent biocompatible.[176] Wang et al.[163] demonstrated the inhibition of *S. mutans* by orthodontic brackets with non-crosslinked chitosan coating for the first time. What is more, another study investigated the impact of adding chitosan nanoparticles on the biofilm formation ability of directly printed clear aligners. The results showed that the addition of chitosan enhanced the antibiofilm efficacy of aligners against *S. mutans* without compromising the cytotoxicity and certain physical and mechanical properties of aligners.[164]

Additionally, a study explored the antimicrobial characteristics of a cellulose-based material loaded with essential oils, such as cinnamaldehyde. Using the isothermal microcalorimetry method, Zhang et al. measured the growth of a bacterial biofilm at the interface between the tested material and the solid growth medium, demonstrating that the addition of cinnamaldehyde reduced microbial growth and plaque biofilm formation.[165] Eskandari et al.[174] fabricated the orthodontic elastomeric ligatures coated with bacterial nanocellulose (BNC) and evaluated its antibacterial ability. The outcomes revealed that the novel ligatures exhibited sustained antimicrobial activity for 28 days. The 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer displayed excellent protein repellent and anti-bacterial adhesion ability. A novel self-etching adhesive modified with MPC was synthesized to enhance its antibacterial properties. The results showed that the novel self-etching orthodontic adhesive had favorable ability to inhibit biofilm formation and reduce enamel demineralization.[175]

In order to improve the corrosion resistance and antibacterial performance of orthodontic composite arch wires, He et al.[177] prepared orthodontic composite arch wires coated with lysozyme. The lysozyme coating improved biocompatibility and endowed the orthodontic composite arch wire surfaces with anti-*Staphylococcus aureus* activity. What is more, bovine serum albumin (BSA), a cheap serum protein, possesses no bactericidal properties but has unique anti-bioadhesion features. The investigation revealed that the stainless steel arch wire modified by BSA molecules could effectively inhibit bacterial adhesion. Similarly, BSA-modified brackets displayed excellent anti-bioadhesion properties.[178]

DISCUSSION

The present work summarizes in detail the effects of different types of orthodontic appliances on the oral microbiome. Based on previous studies, it could be concluded that orthodontic appliance placement could significantly affect the oral microbiome at different ecological niches in the oral cavity, regardless of the types of orthodontic appliances. This effect might be associated with the fact that orthodontic appliances provide a rough surface for bacteria attachment, promoting the retention of plaque, and impeding good oral hygiene.

The insertion of FAs leads to changes in the saliva microbiome and dental plaque microbiome, which might increase the susceptibility of patients to periodontal disease and dental caries. In short-term studies, 3-month studies showed no statistically significant change in *S. mutans* and *Lactobacilli* in the saliva microbiome.[49,55] However, in long-term studies, most researchers have shown that orthodontic treatment significantly increases the *S. mutans* and *Lactobacilli* counts in the saliva of patients. According to previous studies, *S. mutans* and *Lactobacilli* are potential cariogenic bacteria.[66] We can draw a conclusion that long-term fixed orthodontic treatment adversely affects the oral salivary microbiome and has the potential to increase the susceptibility of orthodontic patients to caries.

In addition, some researchers have focused on the influence of FAs on the microbiome of supragingival plaque and subgingival plaque. The results demonstrated that within 3 months of treatment, FAs led to alterations in supragingival plaque and subgingival plaque that were more favorable to the development of

caries and periodontal disease.[48,81] An investigation revealed that 8 literature-identified periodontal and cariogenic pathogens underwent significant changes in supragingival plaque over time.[48] And another study investigated the subgingival microbial alterations in female adult patients wearing FAs within 3 months. At the species level, *Prevotella intermedia*, *Campylobacer rectus*, *Fusobacterium nucleatum*, and *Treponema denticola* elevated mildly, which might indicate that the subgingival plaque microbiome affected by FAs could contribute to the transient mild gingival inflammation.[81]

However, we also found that the microbiological changes induced by FAs were partially reversible. According to a previous study, after only 10 days of orthodontic appliance removal, the subgingival plaque microbiota could be shifted in a healthier direction.[56] At the same time, we also compared the effects of different types of FAs on oral microecology. For better oral health, we prefer the traditional labial wire-ligated appliances with self-ligating brackets and molar bands. Elastomeric rings bonded molar tubes, and the lingual position of the appliances might lead to the deterioration of oral hygiene and have a negative impact on oral health.[87]

Although several studies showed different results, most studies have confirmed that CAs possess more oral health benefits than FAs, and the effects of the CAs on saliva and dental plaque microbiome are also milder.[75,82,102,104] Therefore, only from the perspective of oral health, in clinical practice, we tend to choose CAs with cleaner and more comfortable properties rather than FAs. In addition, other types of orthodontic appliances, including transparent retainers and rapid expansion appliances, also exert a certain impact on the oral microbiome.[58,110,111]

The placement of any type of orthodontic appliance can lead to plaque retention and oral microecological disturbances. Therefore, it is necessary to take some measures to protect the oral microecology during orthodontic treatment. Some researchers have taken measures to improve the antimicrobial properties of orthodontic devices by adding antibacterial materials to orthodontic devices. The antimicrobial materials are divided into four categories: metals and metal compounds, inorganic non-metallic compounds, and organic compounds.

Metals and metal compounds are the most widely applied, mainly including silver and silver compounds, titanium and titanium compounds, zinc and zinc compounds, and gold and gold compounds. Most of the metals and metal compounds have been demonstrated to have a significant inhibitory effect on cariogenic bacteria such as *S. mutans*. And an investigation using AuNPs for modified CAs was also confirmed to have a favorable inhibitory effect on *Porphyromonas gingivalis*. In addition, among the inorganic non-metallic compounds, GO, CHX, fluoride, and BAG showed excellent antimicrobial properties. Organic compounds such as chitosan, cinnamaldehyde, MPC, lysozyme, and BSA all showed good antimicrobial properties in conjunction with orthodontic appliances. In recent years, research on the antimicrobial properties of orthodontic appliances has been abundant, but few targeted studies have been conducted on the inhibition of periodontal pathogens. Such studies are necessary in the context of the realization that various types of orthodontic appliances have the potential to cause adverse changes in periodontal disease-associated microorganisms.

Moreover, these investigations associated with improved antimicrobial properties of orthodontic appliances lack *in vivo* experiments and clinical safety remains to be confirmed. In summary, we hope that our work will provide guidance and direction for further advances in the antimicrobial properties of orthodontic appliances and oral hygiene maintenance in orthodontic patients.

CONCLUSIONS

Orthodontic appliance placement in the mouth can significantly affect the oral microbiota at different ecological niches in the oral cavity, regardless of the types of orthodontic appliances. Fixed orthodontic appliances affect the oral microbiome, which might increase the susceptibility of patients to periodontal disease and caries; however, it is partially reversible after the removal of the FAs. Compared with FAs, CAs have higher oral health benefits, and their effects on saliva and dental plaque microbiome were also milder. Therefore, orthodontic clinicians should strengthen oral health instruction and encourage patients to develop good oral health habits, so as to control plaque accumulation and improve oral health.

In recent years, abundant researches have been implemented to enhance the antimicrobial properties of orthodontic appliances, and the applied materials have been divided into three main classifications: metals and metal compounds, organic compounds, and inorganic non-metallic compounds. However, these investigations have focused more on the inhibition of cariogenic bacteria. Researchers need to explore more effective measures to improve the inhibitory effect of orthodontic appliances on periodontal pathogens and to avoid undesirable changes in the patient's dentition and periodontium during the orthodontic process.

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AUTHOR CONTRIBUTIONS

Conceptualization, Q.N., S.C., T.Y., and B.H.; writing—original draft preparation, Q.N.; writing—review and editing, C.S. and L.P.; validation, supervision, T.Y. and B.H.; project administration, T.Y. and B.H.; funding acquisition, S.C., T.Y., and B.H. All authors have read and agreed to the published version of the article.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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