Concise Review

Impact of Clear Aligners on Oral Health and Oral Microbiome During Orthodontic Treatment

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ABSTRACT

The demand for clear aligners has risen over the past decade because they satisfy patients' desire for less noticeable and more comfortable orthodontic appliances. Because clear aligners are increasingly used in orthodontics, there is a big push to learn more about the physiologic and microbial changes that occur during treatment. The present work highlighted further links between clear aligners and changes in oral health and the oral microbiome and provided plaque control methods for clear aligner trays. Existing literature revealed that clear aligners have no significant influence on the structure of the oral microbiome during orthodontic therapy. Clear aligner treatment demonstrated promising results in terms of controlling plaque index, gingival health, and the prevalence of white spot lesions. Nevertheless, grooves, ridges, microcracks, and abrasions on the aligner surface would provide a prime environment for bacterial adherence and the development of plaque biofilms. A combination of mechanical and chemical methods seems to be a successful approach for removing plaque biofilm from aligners whilst also preventing pigment adsorption.

Introduction

Malocclusion is a common oral health issue that affects not only the function of the orofacial system but also the patients' long-term psychosocial well-being. Orthodontic therapy aims to correct malocclusion and craniofacial skeletal discrepancies whilst also improving mastication and appearance. However, the direct or indirect impacts of orthodontic appliances on the oral microbiome and periodontal tissues must not be overlooked. The insertion of orthodontic appliances alters the structure of plaque biofilm qualitatively.[1-3] Throughout orthodontic treatment, oral hygiene routines have a significant impact on dental and periodontal health.[4-8] The present evidence showed a correlation between decreased oral health and increased plaque indices (PIs) in orthodontic patients with fixed appliances.[9-11] Removable appliances can lessen the detrimental effects of orthodontics on oral health by making oral hygiene procedures easier for patients.[12]

In recent years, a rising number of adult patients have sought orthodontic treatment, expressing a desire for more aesthetically pleasing and comfortable alternatives to traditional fixed equipment.[13] The transparency and low stiffness of the aligners have met the needs of the patients. Furthermore, patients treated with aligners reported less pain and greater quality of life throughout orthodontic treatment.[14]

The evolution of clear aligner therapy has been aided in recent years by the advancement of CAD-CAM technology and transparent thermoplastic materials, as well as increased patient demand for more comfortable and aesthetically pleasing orthodontic appliances.[15] Nowadays, plenty of brands of aligners are available on the market. They differ primarily in forming material, wearing time, gingival margin design (Figure 1), and the presence of attachments and ancillaries.[16] With the introduction of new thermoplastic

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Fig. 1 – Two main gingival margin design features of clear aligners. A, The gingival margin of the aligner is designed as scalloped. B, The gingival margin of the aligner is designed as straight 2 mm up the gingival zenith.

materials, clear aligners have become more flexible, adaptive to the dental arch, and constant in orthodontic force.[17] Over the last 20 years, thermoplastic removable appliances have evolved, and clear aligner treatment techniques have been refined. With the help of extra tools such as mini-screws, elastics, sectional wires, and rapid palatal expanders,[18-22] clear aligners can now be used for more than just simple cases.[15,16]

The goal of this review is to provide a concise and up-to-date overview of the impact of clear aligners on oral health and microbiome during orthodontic treatment as well as to provide cleaning and disinfecting methods for clear aligners.

Methods

A literature search was done in PubMed, Web of Science, and CNKI databases using the keywords "clear aligner," "aligner," "removable appliances," "Invisalign," "oral health," "periodontal health," "caries," "white spot lesions," "periodontitis," "gingivitis," "oral microbiome," and "oral microbiota" to identify the literature published in English and Chinese between January 2015 and August 2022. A total of 393 publications were identified, titles and abstracts were reviewed, duplicate articles were removed, and 100 were investigated out of the 393. The coauthors assessed the titles and abstracts of the papers and chose those that were suitable for inclusion in the full text. Following a discussion, all reviewers agreed on all deleted data, and any disagreements were sent to corresponding authors. Finally, a nonsystematic narrative review of the literature was conducted based on 21 papers. The risk of bias assessment was done on the included meta-analysis and systematic review by combining the proposed criteria of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[23] and Risk of Bias in Systematic Reviews (ROBIS)[24] (Table 1). A 3-point grading system, described by the Swedish Council on Technology Assessment in Health Care (SBU)[25] was used to assess the methodological quality of other selected papers (Table 2) as follows:

1. Grade A (high level of evidence): randomised controlled trials or prospective study with a well-defined control group; presence of defined diagnosis and end points; well-described diagnostic reliability tests and reproducibility tests; blinded outcome assessment.
2. Grade B (moderate value of evidence): cohort study or retrospective case series with defined control or reference group; defined diagnosis and end points; diagnostic reliability tests and reproducibility tests described.
3. Grade C (low level of evidence): articles that do not meet the criteria of grades A and B.

Impact of clear aligners on dental and periodontal health

White spot lesions (WSLs) are characterised as an opaque, milky white region on smooth tooth surfaces in the early stages of caries.[26] Development of WSLs is an intermittent process, with phases of remineralisation and demineralisation dependent on the oral environment, including the amount of plaque formation and how long it remains on the enamel surface, the individual's dental hygiene routine, and their own innate resistance.[27] Orthodontic therapy increases the incidence of WSLs because fixed appliances compromise oral hygiene and increase plaque retention. The removable feature of clear aligners has simplified oral hygiene routines and decreased the occurrence of WSLs. Azeem et al[28] used quantified light-induced fluorescence imaging to examine the incidence of WSL development in 25 participants treated with clear aligners. Their data revealed that the overall incidence of new WSLs was 2.85% for all the assessed teeth. Research conducted by Buschang et al[29] found that patients treated with aligners had a lower risk of experiencing WSLs than patients treated with traditional braces. WSLs formed in just 1.2% of patients using aligners compared with 26% of individuals undergoing conventional treatment. The shorter treatment time and the maintenance of good oral hygiene may contribute to the low incidence of WSLs in patients using clear aligners.[28-30]

The majority of studies on the influence of clear aligner treatment on periodontal health have reached a similar conclusion: Clear aligner treatment is more beneficial to patients' periodontal health maintenance than fixed appliances.[12,31-38] There were no statistically significant changes in bleeding on probing and probing depth of patients using clear aligners in the short or long term.[31,34] Plaque accumulation is the main aetiological factor for gingivitis. Patients treated with clear aligners can clean their teeth and aligners at any time, allowing them to easily eliminate plaque biofilm.

Orthodontic therapy must be based on periodontal health and should conform to the criterion that the treatment should not cause harm to the periodontal tissues. Patients with periodontitis have an increased need to keep their

Table 1 – Risk of bias assessment of the included systematic review and meta-analysis.

Author and year	Theme/focus of the study	Study design	Study eligibility criteria	Identification and selection of studies	Data collection and study appraisal	Synthesis and findings	Potential risk of bias
Jiang et al[12] 2018	Periodontal health status of patients with clear aligners and patients with fixed appliances	Meta-analysis	Yes	Yes	Yes	Yes	Low
Rossini et al[35] 2015	Periodontal health during clear aligners treatment	Systematic review	Yes	Yes	Yes	Yes	Low
Lu et al[36] 2018	Periodontal health status of patients with clear aligners and patients with fixed appliances	Meta-analysis	Yes	Yes	Yes	Yes	Low
Wu et al[37] 2020	Periodontal health status of patients with clear aligners and patients with fixed appliances	Meta-analysis	Yes	Yes	Yes	Yes	Low
Oikonomou et al[38] 2021	Impact of clear aligners and fixed appliances on oral health	Systematic review and meta-analysis	Yes	Yes	Yes	Yes	Low

periodontal tissues healthy and free from additional damage or periodontitis recurrence throughout orthodontic therapy. On the one hand, aligners make plaque control easier for patients, reducing plaque buildup and avoiding the worsening of periodontitis.[12] Clear aligners, on the other hand, produce regulated intermittent forces during treatment time, providing for a specific interval for periodontal membrane rebuilding.[39] Clear aligners provide a more even stress distribution. Using 3-dimensional finite element analysis, researchers observed that when aligners were used, the strains on the teeth and alveolar bone were more equally distributed, with fewer areas of stress concentration.[40,41] Lee et al[42] employed clear aligners to treat 3 patients with chronic periodontal disease combined with maxillary anterior pathologic tooth migration after a series of case-related periodontal treatments and observed a favourable treatment outcome. They discovered a decrease in the probing depth, gingival recession, clinical attachment level, and mobility during treatment. Han et al[43] investigated the impact of 2 types of orthodontic appliances on periodontal tissue in patients with periodontitis. Patients with clear aligners had relatively good orthodontic treatment outcomes, and the periodontitis did not worsen. Patients' plaque index, gingival index, probing depth, and bone level improved after orthodontic treatment.

In conclusion, clear aligners are preferred by both practitioners and patients for their convenience, comfort, and benefit to periodontal health maintenance.[12,31,33] According to current studies, clear aligners can provide good treatment outcomes for patients with periodontitis whilst preventing additional periodontal aggravation. However, most of these are case reports, with no randomised controlled trials and long-term follow-up. High-quality research is required to make a conclusive recommendation.

Impact of clear aligners on the oral microbiome

The oral microbiome is defined as a collection of more than 700 microbial species that live in 3 distinct microbial metaniches: group 1, sub- and supragingival plaque; group 2, saliva, tongue, and hard palate; and group 3, cheek and sublingual area.[44] Through a dynamic balance, the oral microbiome contributes to the host's local and overall health. Any considerable change to the local environment may affect the host–microbe balance, increasing the risk of oral diseases.[45] This microbial shift is a major cause of dental and periodontal disorders, such as WSLs, caries, and gingivitis. Therefore, maintaining the stability and equilibrium of the plaque biofilm is critical for effective treatment completion during orthodontic therapy.

Changes in the microbiological structure of supragingival plaque, subgingival plaque, aligner plaque, and saliva were the major focus of studies on the impact of clear aligners on oral flora. The research methods are mainly divided into real-time polymerase chain reaction (PCR) analysis, checkerboard DNA-DNA hybridisation (CDDH), and 16S rRNA gene sequencing (Figure 2). The PCR and CDDH methods, with their quantitative and qualitative potential, are accurate and useful approaches for characterising the oral microbiome.[46,47] The CDDH molecular approach employs several DNA probes concurrently and permits the simultaneous determination of different bacterial species in a single or many samples.[48] 16S rRNA gene

Table 2 – Quality grading of the selected papers

Author and year	Theme/focus of the study	Study design	Population	Outcome measurements	Quality of the evidence (SBU grading system)
Azeem et al[28] 2017	Incidence of white spot lesions during clear aligner therapy	Retrospective cohort study	25 clear aligner	White spot lesions	B
Buschang et al[29] 2018	Incidence of white spot lesions amongst patients treated with clear aligners and fixed appliances	Retrospective cohort study	244 clear aligner 206 fixed appliance	Oral hygiene White spot lesions Treatment duration	B
Azaripour et al[31] 2015	Oral health status, oral hygiene, and patients' satisfaction with aligners and fixed appliances	Cross-sectional study	50 clear aligner 50 fixed appliance	Gingival index Sulcus bleeding index Approximal plaque index Oral hygiene habits	C
Madariaga et al[32] 2020	Impact of fixed appliances and clear aligners on periodontal health	Prospective cohort study	20 clear aligner 20 fixed appliance	Probing depth Plaque index Bleeding on probing Gingival recession	B
Abbate et al[33] 2015	Microbiological and periodontal changes occurring in adolescents treated with clear aligners and fixed appliances	Randomised clinical trial	25 clear aligner 25 fixed appliance	Periodontal pathogens Probing depth Plaque index Bleeding on probing Full mouth plaque score Full mouth bleeding score Compliance with oral hygiene	A
Levrini et al[34] 2015	Microbiological and periodontal changes occurring in clear aligner and fixed appliance treatment	Randomised clinical trial	10 untreated control 32 clear aligner 35 fixed appliance	Probing depth Plaque index Bleeding on probing Periodontal bacteria quantification	A
Han et al[43] 2015	Impact of fixed appliances and clear aligners on the periodontal health in patients with periodontitis	Prospective cohort study	16 clear aligner 19 fixed appliance	Probing depth Plaque index Gingival index Treatment duration	B
Guo et al[49] 2018	Impact of clear aligners on subgingival plaque biofilm	Prospective study	10 clear aligner	Plaque index Gingival bleeding index Alpha diversity Beta diversity Microbial distribution and relative abundances	C
Shokeen et al[51] 2022	Impact of fixed orthodontic appliances and clear aligners on the oral microbiome and the association with clinical parameters	Prospective cohort study	12 clear aligner 12 fixed appliance	Plaque index Gingival index Alpha diversity Beta diversity Microbial distribution and relative abundances	B

(*continued*)

Table 2 (*Continued*)

Author and year	Theme/focus of the study	Study design	Population	Outcome measurements	Quality of the evidence (SBU grading system)
Yan et al[53] 2021	Changes in the microbiome of the inner surface of clear aligner	Prospective study	8 clear aligner	pH of saliva Alpha diversity Microbial distribution and relative abundances	C
Gujar et al[54] 2020	Prevalence of orange and red microbial complexes in patients treated with clear aligners and fixed appliances	Prospective cohort study	20 clear aligner 40 fixed appliance	Appearance of red and orange microorganisms	A
Sfondrini et al[58] 2021	Changes of periodontal status and microbiological composition in patients with clear aligners	Randomised clinical trial	23 untreated control 21 clear aligner	Probing depth Plaque index Bleeding on probing Appearance of red and orange microorganisms	A
Lombardo et al[59] 2021	Variation in the subgingival microbiota in patients with clear aligners and patients with fixed appliances	Prospective cohort study	14 clear aligner 13 fixed appliance	Total bacterial load	B
Mummolo et al[61] 2020	Salivary concentrations of *Streptococcus mutans* and Lactobacilli	Prospective cohort study	30 clear aligner 30 fixed appliance 30 removable positioner	Concentrations of *S mutans* and Lactobacilli Plaque index	A
Mummolo et al[62] 2020	Salivary levels of *Streptococcus mutans* and Lactobacilli and other salivary indices	Prospective cohort study	40 clear aligner 40 fixed appliance	Concentrations of *S mutans* and Lactobacilli Plaque index Salivary flow Salivary buffering power	A
Zhao et al[63] 2020	The dynamics of the salivary microbiome and oral health	Prospective study	25 clear aligner	Probing depth Plaque index Bleeding on probing Oral hygiene habits Alpha diversity Beta diversity Microbial distribution and relative abundances	C

SBU, Swedish Council on Technology Assessment in Health Care.

sequencing determines the complete bacterial genome in a single sequence run and provides a more sensitive and complete amount of data on microbial diversity (including unculturable bacteria) and community shifts, allowing taxonomic analysis and comparison of bacterial composition across samples.[49,50]

The changes of supra- and subgingival microbiome during clear aligner treatment

Shokeen et al[51] used 16S rRNA gene sequencing to investigate the alterations in the microbial community composition of patients with clear aligners. Plaque samples were collected from the supragingival surfaces of anterior and posterior teeth as well as from the inner surfaces of aligners. Their study found no significant differences in the microbial community composition of tooth-associated plaque over time. The plaque collected from clear aligner trays, on the other hand, contained a distinct and less diversified community with higher levels of *Streptococcus* and *Granulicatella*, which were previously identified as caries-associated bacteria by Peterson et al.[52] Research conducted by Yan et al[53] also discovered a reduction in microbial abundance and an increase in *Streptococcus* abundance in aligner plaque samples. Gujar et al[54] used the CDDH approach to assess the extent of the emergence of orange and red microbial complexes in aligners,

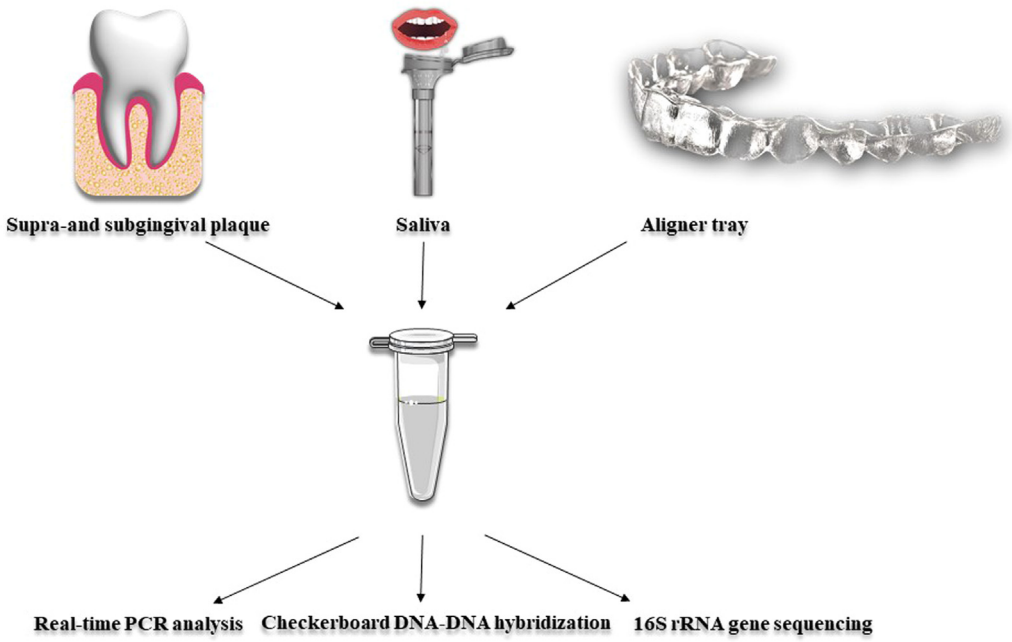

Fig. 2 – Sample sources and investigating methods of oral microbiome during clear aligner treatment.

conventional metallic fixed labial appliances, and lingual fixed appliances after 1 month of therapy. Compared with the other 2 appliances, aligners revealed less microbial colonisation and a lower quantity of red and orange complexes.

Although clear aligners will not bring significant alteration to supragingival plaque, the richness and evenness of the tray microbiome will decrease over time. Bacterial species' affinities to the biomaterials used in clear aligners result in an increase in caries-associated bacteria. Furthermore, clear aligners form a completely enclosed environment on crowns. As a result, if patients do not regularly clean their aligners, the environment of the inner surface of aligners may have a negative impact on enamel health. Thus, scholars have coated antibacterial nanoparticles and cinnamaldehyde onto the aligners and tested their capacity to suppress bacterial growth and biofilm formation on the inside surface of aligners.[55-57] In in vitro studies, these antibiotic reagents demonstrated a great ability to inhibit biofilm formation growth of *Streptococcus mutans*, *Streptococcus mitis*, and *Porphyromonas gingivalis*. However, further studies including clinical trials are needed to fully understand the effects of aligners coated with antibacterial materials on oral health and composition of the oral microbiome.

Sfondrini et al[58] employed real-time PCR to compare the changes in total bacteria load (TBL) and periodontal pathogens between patients with clear aligners and non-orthodontic patients. They discovered that the changes in TBL between the 2 groups were not statistically significant and the proportion of red complexes (*Porphyromonas gingivalis*, *Troponema denticola*, and *Tannerella forsythensys*) and orange complexes (*Prevotella intermedia* and *Fusobacterium nucleatum*) did not change significantly. In a previous study, the patients with clear aligners had a statistically significant decreased TBL after 3 months compared to the start of orthodontic treatment.[34] Using the same procedure, Lombardo et al[59] assessed the subgingival microbiological alterations of orthodontic patients over the first 6 months of treatment. Their findings were comparable to those of Sfondrini et al, in that the TBL of patients with clear aligners did not change considerably over time. However, *Campilobacter rectus*, a member of the orange complex, increased over the treatment time. Only one study, conducted by Guo et al,[49] used 16S rRNA gene sequencing technology to assess the impact of clear aligners on the subgingival plaque microbiome. During the first 3 months of treatment, their findings demonstrated a shift in the subgingival microbial community with decreased microbial diversity. However, no significant changes in the relative abundance of periodontal pathogens were observed.

The changes in the salivary microbiome during clear aligner treatment

Dental plaque formation on healthy tooth enamel begins with the attachment of a selected bacteria to the saliva-bathed surface and progresses to the attachment of a greater diversity of salivary bacterial taxa attach to form dental plaque.[60] Saliva also seeps into the spaces between the aligner and the teeth, contributing to plaque buildup on the inner surface of aligners. Additionally, salivary microbes spread into the subgingival pocket, supporting the development of subgingival plaque.[49] As a result, maintaining a healthy oral cavity throughout clear aligner treatment necessitates a steady salivary flora structure.

Mummolo et al[61] measured the *Streptococcus mutans* and *Lactobacilli* count in the saliva of patients receiving clear

aligner therapy by using CRT bacteria. The study's findings showed that amongst 30 participants, 3 and 4 patients, respectively, had a high caries risk level for Streptococci and Lactobacilli in their saliva after 6 months of therapy. In another study, Mummolo et al[62] reached a similar conclusion. After 6 months of clear aligner treatment, 3 patients and 1 patient, respectively, amongst the 40 participants, had a high caries risk level for *Streptococci* and *Lactobacilli* in their saliva. Zhao et al[63] used the 16S rRNA gene sequencing approach to examine the changes in structure and composition of salivary microbial communities to investigate the effects of clear aligners on the saliva microbiome. They discovered that the general biodiversity and salivary microbial community structure did not change significantly during the first 6 months of Invisalign treatment and the relative abundance of *Streptococcus mutans* and *Streptococcus sobrinus* did not change significantly during the treatment time. These findings indicated that during the early phases of clear aligner therapy, the salivary flora may retain a relatively stable structure and the quantity of cariogenic bacteria does not considerably increase. However, it is unclear whether the salivary microbiome can remain relatively stable during the entire process of clear aligner treatment. More research should be conducted with patients from various countries and over a longer period of time, encompassing the complete term of orthodontic therapy.

Cleaning and disinfecting methods for clear aligners

Generally, patients are instructed to wear their aligners at all times, with the exception of eating, brushing, and flossing of their teeth. If patients do not adhere to strict guidelines and consume colouring agents whilst wearing their appliances, this will result in a change in the colour of aligners.[64] Consequently, even throughout the 2-week treatments, transparent aligners may appear less attractive. Clear aligners are not perfectly smooth and contain grooves, ridges, microcracks, and abrasions that encourage bacterial adhesion and the formation of plaque biofilm. Low et al[65] found that even a brand new aligner's surface has a tendency to be corrugated, exhibiting scratch marks, microabrasions, and peaks. These irregularities provide the basis for bacterial attachment and growth. Additionally, Gracco et al[66] found that after 14 days of use, aligners had microcracks, abrasions, and delaminated patches that were conducive to bacterial adhesion and development, as well as localised calcified biofilm deposits and a loss of transparency. When Schuster et al[67] examined older intraoral aligners, they discovered abrasion at the cusp points, integument adsorption, and localised calcification of the precipitated biofilm at stagnation areas. Plaque biofilm accumulates around the teeth when aligners are worn, and they also inhibit the buffering, cleansing, and remineralising benefits of saliva.[68] The formation of biofilm not only affects the appearance of clear aligners but also increases the risk of the emergence of caries and gingivitis.

Effective cleaning methods can prevent plaque accumulation and pigmentation of clear aligners. There are various strategies available for aligner cleaning, including rinsing in cold running water, brushing with toothpaste and toothbrush, and soaking in cold water with detergent. Levrini et al[69] investigated the efficacy of 3 different techniques for eliminating bacterial biofilm from clear aligners. Their study results demonstrated that brushing combined with the use of sodium carbonate and sulfate crystals offered the best cleaning results, whereas brushing alone generated somewhat worse results. Lombardo et al[68] explored the effectiveness of 9 different strategies for removing bacterial biofilm from the inner surface of aligners and discovered that 5 minutes of ultrasonication at 42 kHz combined with a 0.3% germicidal cationic detergent was statistically effective in removing bacterial biofilm from aligners. Research conducted by Bernard et al[70] found that cleaning crystals and sonic cleaners combined with cleaning tablets were effective in eliminating stains from aligners. In particular, washing with water is insufficient to clean the aligners, and mechanical or chemical techniques are required for better cleaning outcomes. Although determining the most effective cleaning and disinfection method is impossible due to the lack of a direct comparison of all these approaches, a multistep procedure that includes both mechanical and chemical methods appears to be the most successful strategy.

Conclusions

The removable nature of clear aligners makes oral hygiene procedures easier and more effective for orthodontic patients. Patients must use a multistep cleaning and disinfection technique that combines mechanical and chemical methods in order to keep aligners bright and clean and prevent biofilm infection. The results of our study indicated that clear aligners may be beneficial for maintaining good oral hygiene and dental and periodontal health. To support this finding, further prospective studies or randomised controlled trials with large sample sizes are required. To fully understand the impact of clear aligners on oral health and oral microbiome structure, the experimental design must encompass all phases of orthodontic treatment, including the early, middle, and late phases as well as the maintenance phase.

Author contributions

Maierdanjiang Rouzi assessed the titles and abstracts of the papers, and wrote and revised the manuscript. Xiaoqi Zhang assessed the titles and abstracts of the papers and performed the risk of bias assessment. Qingsong Jiang assessed the titles and abstracts of the papers and assessed the methodological quality of selected papers. Hu Long assessed the titles and abstracts of the papers. Xiaolong Li and Wenli Lai contributed to the conception of the study.

Conflict of interest

None disclosed.

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REFERENCES

1. Petti S, Barbato E, Simonetti DA. Effect of orthodontic therapy with fixed and removable appliances on oral microbiota: a six-month longitudinal study. New Microbiol 1997;20(1):55–62.
2. Campobasso A, Lo ME, Battista G, Ciavarella D, Crincoli V, Lo ML. Taxonomic analysis of oral microbiome during orthodontic treatment. Int J Dent 2021;2021:8275181.
3. Ireland AJ, Soro V, Sprague SV, et al. The effects of different orthodontic appliances upon microbial communities. Orthod Craniofac Res 2014;17(2):115–23.
4. Alves KM, Goursand D, Zenobio EG, Cruz RA. Effectiveness of procedures for the chemical-mechanical control of dental biofilm in orthodontic patients. J Contemp Dent Pract 2010;11 (2):41–8.
5. Karadas M, Cantekin K, Celikoglu M. Effects of orthodontic treatment with a fixed appliance on the caries experience of patients with high and low risk of caries. J Dent Sci 2011;6 (4):195–9.
6. Marya A, Steier L, Karobari MI, Venugopal A. Benefits of using fluorescence induced theragnosis in fixed orthodontic therapy: status, technology and future trends. Dent J (Basel) 2021;9(8).
7. Lundstrom F, Hamp SE, Nyman S. Systematic plaque control in children undergoing long-term orthodontic treatment. Eur J Orthod 1980;2(1):27–39.
8. Lopes Dos Santos R, Spinola MDS, Carvalho E, Lopes Dos Santos DC, Dame-Teixeira N, Heller D. Effectiveness of a new app in improving oral hygiene in orthodontic patients: a pilot study. Int Dent J 2022.
9. Kaygisiz E, Uzuner FD, Yuksel S, et al. Effects of self-ligating and conventional brackets on halitosis and periodontal conditions. Angle Orthod 2015;85(3):468–73.
10. SA Al-Anezi. Dental plaque associated with self-ligating brackets during the initial phase of orthodontic treatment: a 3-month preliminary study. J Orthod Sci 2014;3(1):7–11.
11. Boke F, Gazioglu C, Akkaya S, Akkaya M. Relationship between orthodontic treatment and gingival health: a retrospective study. Eur J Dent 2014;8(3):373–80.
12. Jiang Q, Li J, Mei L, et al. Periodontal health during orthodontic treatment with clear aligners and fixed appliances: a meta-analysis. J Am Dent Assoc 2018;149(8):712–20.
13. Rossini G, Parrini S, Castroflorio T, Deregibus A, Debernardi CL. Efficacy of clear aligners in controlling orthodontic tooth movement: a systematic review. Angle Orthod 2015;85(5):881–9.
14. Miller KB, McGorray SP, Womack R, et al. A comparison of treatment impacts between Invisalign aligner and fixed appliance therapy during the first week of treatment. Am J Orthod Dentofacial Orthop 2007;131(3):301–2.
15. Weir T. Clear aligners in orthodontic treatment. Aust Dent J 2017;62(Suppl 1)):58–62.
16. Putrino A, Barbato E, Galluccio G. Clear aligners: between evolution and efficiency-a scoping review. Int J Environ Res Public Health 2021;18(6).
17. Condo R, Pazzini L, Cerroni L, et al. Mechanical properties of "two generations" of teeth aligners: Change analysis during oral permanence. Dent Mater J 2018;37(5):835–42.
18. Lombardo L, Colonna A, Carlucci A, Oliverio T, Siciliani G. Class II subdivision correction with clear aligners using intermaxilary elastics. Prog Orthod 2018;19(1):32.
19. Liu Y, Hu W. Force changes associated with different intrusion strategies for deep-bite correction by clear aligners. Angle Orthod 2018;88(6):771–8.
20. Caruso S, Nota A, Ehsani S, Maddalone E, Ojima K, Tecco S. Impact of molar teeth distalization with clear aligners on occlusal vertical dimension: a retrospective study. BMC Oral Health 2019;19(1).
21. Kankam H, Madari S, Sawh-Martinez R, Bruckman KC, Steinbacher DM. Comparing outcomes in orthognathic surgery using clear aligners versus conventional fixed appliances. J Craniofac Surg 2019;30(5):1488–91.
22. Kook M, Kim H, Oh H, Lee K. Clear aligner use following surgery-first mandibular prognathism correction. J Craniofac Surg 2019;30(6):E544–7.
23. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. PLoS Med 2021;18(3):e1003583.
24. Whiting P, Savović J, Higgins JPT, et al. ROBIS: a new tool to assess risk of bias in systematic reviews was developed. J Clin Epidemiol 2016;69:225–34.
25. Bondemark L, Holm AK, Hansen K, et al. Long-term stability of orthodontic treatment and patient satisfaction - a systematic review. Angle Orthod 2007;77(1):181–91.
26. Sundararaj D, Venkatachalapathy S, Tandon A, Pereira A. Critical evaluation of incidence and prevalence of white spot lesions during fixed orthodontic appliance treatment: a meta-analysis. J Int Soc Prev Community Dent 2015;5(6):433–9.
27. Sudjalim TR, Woods MG, Manton DJ. Prevention of white spot lesions in orthodontic practice: a contemporary review. Aust Dent J 2006;51(4):284–9.
28. Azeem M, Ul Hamid W. Incidence of white spot lesions during orthodontic clear aligner therapy. J World Fed Orthod 2017;6 (3):127–30.
29. Buschang PH, Chastain D, Keylor CL, Crosby D, Julien KC. Incidence of white spot lesions among patients treated with clear aligners and traditional braces. Angle Orthod 2018;89(3):359–64.
30. Buschang PH, Shaw SG, Ross M, Crosby D, Campbell PM. Comparative time efficiency of aligner therapy and conventional edgewise braces. Angle Orthod 2014;84(3):391–6.
31. Azaripour A, Weusmann J, Mahmoodi B, et al. Braces versus Invisalign®: gingival parameters and patients' satisfaction during treatment: a cross-sectional study. BMC Oral Health 2015;15:69.
32. Madariaga A, Bucci R, Rongo R, Simeon V, D'Antò V, Valletta R. Impact of fixed orthodontic appliance and clear aligners on the periodontal health: a prospective clinical study. Dent J (Basel) 2020;8(1).
33. Abbate GM, Caria MP, Montanari P, et al. Periodontal health in teenagers treated with removable aligners and fixed orthodontic appliances. J Orofac Orthop 2015;76(3):240–50.
34. Levrini L, Mangano A, Montanari P, Margherini S, Caprioglio A, Abbate GM. Periodontal health status in patients treated with the Invisalign(®) system and fixed orthodontic appliances: a 3 months clinical and microbiological evaluation. Eur J Dent 2015;9(3):404–10.
35. Rossini G, Parrini S, Castroflorio T, Deregibus A, Debernardi CL. Periodontal health during clear aligners treatment: a systematic review. Eur J Orthodont 2015;37(5):539–43.
36. Lu H, Tang H, Zhou T, Kang N. Assessment of the periodontal health status in patients undergoing orthodontic treatment with fixed appliances and Invisalign system: a meta-analysis. Medicine (Baltimore) 2018;97(13):e248.

37. Wu Y, Cao L, Cong J. The periodontal status of removable appliances vs fixed appliances: a comparative meta-analysis. Medicine (Baltimore) 2020;99(50):e23165.
38. Oikonomou E, Foros P, Tagkli A, Rahiotis C, Eliades T, Koletsi D. Impact of aligners and fixed appliances on oral health during orthodontic treatment: a systematic review and meta-analysis. Oral Health Prev Dent 2021;19(1):659–72.
39. Wu J, Hou J. Influences on periondtal health and conditions of patients with periodontitis by clear aligner treatment. Chin J Stomatol 2019;54(1):62–6.
40. Yang B, Bai Y. The comparision of the stress distribution in the PDL of the patients with the periodontitis by using the two different appliances. J Mod Stomatol 2009;23(03):232–4.
41. Dou N, Guo XN, He BW. Biomechanics experimental analysis on dental cast under orthodontic forces with invisaligner. J Med Biomech 2008;23(06):454–8.
42. Lee J, Lee S, Lee C, Kim B. Orthodontic treatment for maxillary anterior pathologic tooth migration by periodontitis using clear aligner. J Periodontal Implant Sci 2011;41(1):44.
43. Han JY. A comparative study of combined periodontal and orthodontic treatment with fixed appliances and clear aligners in patients with periodontitis. J Periodontal Implant Sci 2015;45(6):193–204.
44. Seidel CL, Gerlach RG, Wiedemann P, et al. Defining metaniches in the oral cavity according to their microbial composition and cytokine profile. Int J Mol Sci 2020;21(21):8218.
45. Gao L, Xu TS, Huang G, Jiang S, Gu Y, Chen F. Oral microbiomes: more and more importance in oral cavity and whole body. Protein Cell 2018;9(5):488–500.
46. Andrucioli M, Nelson P, Matsumoto M, et al. Molecular detection of in-vivo microbial contamination of metallic orthodontic brackets by checkerboard DNA-DNA hybridization. Am J Orthod Dentofacial Orthop 2012;141(1):24–9.
47. Lombardo L, Palone M, Scapoli L, Siciliani G, Carinci F. Short-term variation in the subgingival microbiota in two groups of patients treated with clear aligners and vestibular fixed appliances: a longitudinal study. Orthod Craniofac Res 2021;24(2):251–60.
48. Haffajee AD, Arguello EI, Ximenez-Fyvie LA, Socransky SS. Controlling the plaque biofilm. Int Dent J 2003;53:191–9.
49. Guo R, Zheng Y, Liu H, Li X, Jia L, Li W. Profiling of subgingival plaque biofilm microbiota in female adult patients with clear aligners: a three-month prospective study. PeerJ 2018;6:e4207.
50. Campobasso A, Lo Muzio E, Battista G, Ciavarella D, Crincoli V, Lo Muzio L. Taxonomic analysis of oral microbiome during orthodontic treatment. Int J Dent 2021;2021:1–12.
51. Shokeen B, Viloria E, Duong E, et al. The impact of fixed orthodontic appliances and clear aligners on the oral microbiome and the association with clinical parameters: a longitudinal comparative study. Am J Orthod Dentofacial Orthop 2022.
52. Peterson SN, Snesrud E, Liu J, et al. The dental plaque microbiome in health and disease. PLoS One 2013;8(3):e58487.
53. Yan D, Liu Y, Che X, et al. Changes in the microbiome of the inner surface of clear aligners after different usage periods. Curr Microbiol 2021;78(2):566–75.
54. Gujar AN, Al-Hazmi A, Raj AT, Patil S. Microbial profile in different orthodontic appliances by checkerboard DNA-DNA hybridization: an in-vivo study. Am J Orthod Dentofacial Orthop 2020;157(1):49–58.
55. Worreth S, Bieger V, Rohr N, et al. Cinnamaldehyde as antimicrobial in cellulose-based dental appliances. J Appl Microbiol 2022;132(2):1018–24.
56. Xie Y, Zhang M, Zhang W, Liu X, Zheng W, Jiang X. Gold nanoclusters-coated orthodontic devices can inhibit the formation of *Streptococcus mutans* biofilm. ACS Biomater Sci Eng 2020;6(2):1239–46.
57. Zhang M, Liu X, Xie Y, et al. Biological safe gold nanoparticle-modified dental aligner prevents the *Porphyromonas gingivalis* biofilm formation. ACS Omega 2020;5(30):18685–92.
58. Sfondrini MF, Butera A, Di Michele P, et al. Microbiological changes during orthodontic aligner therapy: a prospective clinical trial. Appl Sci 2021;11(15):6758.
59. Lombardo L, Palone M, Scapoli L, Siciliani G, Carinci F. Short-term variation in the subgingival microbiota in two groups of patients treated with clear aligners and vestibular fixed appliances: a longitudinal study. Orthod Craniofac Res 2021;24(2):251–360.
60. Yasunaga H, Takeshita T, Shibata Y, et al. Exploration of bacterial species associated with the salivary microbiome of individuals with a low susceptibility to dental caries. Clin Oral Investig 2017;21(8):2399–406.
61. Mummolo S, Tieri M, Nota A, et al. Salivary concentrations of *Streptococcus mutans* and Lactobacilli during an orthodontic treatment. An observational study comparing fixed and removable orthodontic appliances. Clin Exp Dent Res 2020;6(2):181–7.
62. Mummolo S, Nota A, Albani F, et al. Salivary levels of *Streptococcus mutans* and Lactobacilli and other salivary indices in patients wearing clear aligners versus fixed orthodontic appliances: an observational study. PLoS One 2020;15(4):e228798.
63. Zhao R, Huang R, Long H, Li Y, Gao M, Lai W. The dynamics of the oral microbiome and oral health among patients receiving clear aligner orthodontic treatment. Oral Dis 2020;26(2):473–83.
64. Porojan L, Vasiliu RD, Porojan SD, Bîrdeanu MI. Surface quality evaluation of removable thermoplastic dental appliances related to staining beverages and cleaning agents. Polymers (Basel) 2020;12(8).
65. Low B, Lee W, Seneviratne CJ, Samaranayake LP, Hägg U. Ultrastructure and morphology of biofilms on thermoplastic orthodontic appliances in 'fast' and 'slow' plaque formers. Eur J Orthod 2011;33(5):577–83.
66. Gracco A, Mazzoli A, Favoni O, et al. Short-term chemical and physical changes in invisalign appliances. Aust Orthod J 2009;25(1):34–40.
67. Schuster S, Eliades G, Zinelis S, Eliades T, Bradley TG. Structural conformation and leaching from in vitro aged and retrieved Invisalign appliances. Am J Orthod Dentofacial Orthop 2004;126(6):725–8.
68. Lombardo L, Martini M, Cervinara F, Spedicato GA, Oliverio T, Siciliani G. Comparative SEM analysis of nine F22 aligner cleaning strategies. Prog Orthod 2017;18(1).
69. Levrini L, Novara F, Margherini S, Tenconi C, Raspanti M. Scanning electron microscopy analysis of the growth of dental plaque on the surfaces of removable orthodontic aligners after the use of different cleaning methods. Clin Cosmet Investig Dent 2015;7:125–31.
70. Bernard G, Rompré P, Tavares JR, Montpetit A. Colorimetric and spectrophotometric measurements of orthodontic thermoplastic aligners exposed to various staining sources and cleaning methods. Head Face Med 2020;16(1):2.