

## 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.

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## 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.<sup>1-3</sup> Throughout orthodontic treatment, oral hygiene routines have a significant impact on dental and periodontal health.<sup>4-8</sup> The present evidence showed a correlation

between decreased oral health and increased plaque indices (PIs) in orthodontic patients with fixed appliances.<sup>9-11</sup> Removable appliances can lessen the detrimental effects of orthodontics on oral health by making oral hygiene procedures easier for patients.<sup>12</sup>

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.<sup>13</sup> 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.<sup>14</sup>

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.<sup>15</sup> 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.<sup>16</sup> 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.<sup>17</sup> 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,<sup>18-22</sup> clear aligners can now be used for more than just simple cases.<sup>15,16</sup>

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<sup>23</sup> and Risk of Bias in Systematic Reviews (ROBIS)<sup>24</sup> (Table 1). A 3-point grading system, described by the Swedish Council on Technology Assessment in Health Care (SBU)<sup>25</sup> 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.<sup>26</sup> 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.<sup>27</sup> 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<sup>28</sup> 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<sup>29</sup> 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.<sup>28-30</sup>

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.<sup>12,31-38</sup> There were no statistically significant changes in bleeding on probing and probing depth of patients using clear aligners in the short or long term.<sup>31,34</sup> 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 <sup>12</sup> 2018	Periodontal health status of patients with clear aligners and patients with fixed appliances	Meta-analysis	Yes	Yes	Yes	Yes	Low
Rossini et al <sup>35</sup> 2015	Periodontal health during clear aligners treatment	Systematic review	Yes	Yes	Yes	Yes	Low
Lu et al <sup>36</sup> 2018	Periodontal health status of patients with clear aligners and patients with fixed appliances	Meta-analysis	Yes	Yes	Yes	Yes	Low
Wu et al <sup>37</sup> 2020	Periodontal health status of patients with clear aligners and patients with fixed appliances	Meta-analysis	Yes	Yes	Yes	Yes	Low
Oikonomou et al <sup>38</sup> 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.<sup>12</sup> Clear aligners, on the other hand, produce regulated intermittent forces during treatment time, providing for a specific interval for periodontal membrane rebuilding.<sup>39</sup> 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.<sup>40,41</sup> Lee et al<sup>42</sup> 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<sup>43</sup> 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.<sup>12,31,33</sup> 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 niches: group 1, sub- and supragingival plaque; group 2, saliva, tongue, and hard palate; and group 3, cheek and sublingual area.<sup>44</sup> 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.<sup>45</sup> 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.<sup>46,47</sup> The CDDH molecular approach employs several DNA probes concurrently and permits the simultaneous determination of different bacterial species in a single or many samples.<sup>48</sup> 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 <sup>28</sup> 2017	Incidence of white spot lesions during clear aligner therapy	Retrospective cohort study	25 clear aligner	White spot lesions	B
Buschang et al <sup>29</sup> 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 <sup>31</sup> 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 <sup>32</sup> 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 <sup>33</sup> 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 <sup>34</sup> 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 <sup>43</sup> 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 <sup>49</sup> 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 <sup>51</sup> 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 <sup>53</sup> 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 <sup>54</sup> 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 <sup>58</sup> 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 <sup>59</sup> 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 <sup>61</sup> 2020	Salivary concentrations of <i>Streptococcus mutans</i> and Lactobacilli	Prospective cohort study	30 clear aligner 30 fixed appliance 30 removable positioner	Concentrations of <i>S mutans</i> and Lactobacilli Plaque index	A
Mummolo et al <sup>62</sup> 2020	Salivary levels of <i>Streptococcus mutans</i> and Lactobacilli and other salivary indices	Prospective cohort study	40 clear aligner 40 fixed appliance	Concentrations of <i>S mutans</i> and Lactobacilli Plaque index Salivary flow Salivary buffering power	A
Zhao et al <sup>63</sup> 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

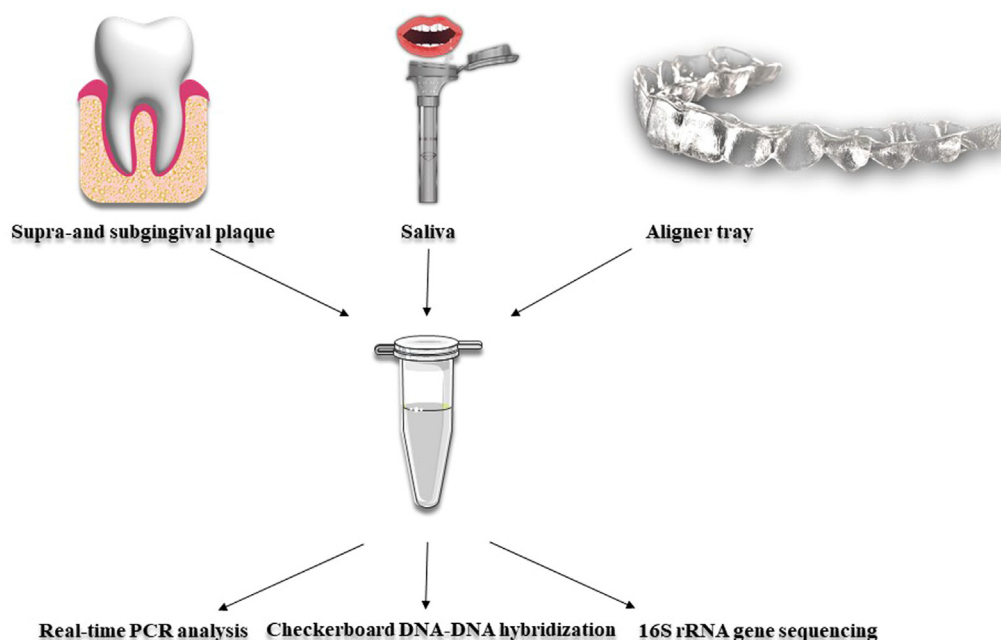
SBU, Swedish Council on Technology Assessment in Health Care.

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.<sup>49,50</sup>

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

Shokeen et al<sup>51</sup> 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.<sup>52</sup> Research conducted by Yan et al<sup>53</sup> also discovered a reduction in microbial abundance and an increase in *Streptococcus* abundance in aligner plaque samples. Gujar et al<sup>54</sup> 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.<sup>55-57</sup> 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<sup>58</sup> 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 forsythensis*) 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.<sup>34</sup> Using the same procedure, Lombardo et al<sup>59</sup> 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,<sup>49</sup> 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.<sup>60</sup> 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.<sup>49</sup> As a result, maintaining a healthy oral cavity throughout clear aligner treatment necessitates a steady salivary flora structure.

Mummolo et al<sup>61</sup> 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<sup>62</sup> 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<sup>63</sup> 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.<sup>54</sup> 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<sup>65</sup> 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<sup>66</sup> 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<sup>67</sup> 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.<sup>68</sup> 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<sup>69</sup> 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<sup>68</sup> 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<sup>70</sup> 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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