Asian Journal of Periodontics and Orthodontics
2025, Volume 5, Page No: 33-42
Copyright CC BY-NC-SA 4.0
Available online at: www.tsdp.net

ISSN: 3062-3499

Review Article

Oral Biofilm Control in Patients Using Orthodontic Aligners: Evidence from a Systematic Review

Carina Bona[1], Fabio Camacho-Alonso[1]*, Andrea Vaca[1], Marta Llorente-Alonso[1,2]

[1]Department of Surgery, Faculty of Medicine and Dentistry, University of Salamanca, 37008 Salamanca, Spain.
[2]Department of Implant Surgery, Faculty of Health Sciences, Alfonso X el Sabio University, 28691 Madrid, Spain.

***E-mail** ✉ alonsofabio@outlook.com

Received: 02 December 2024; **Revised:** 28 February 2025; **Accepted:** 02 March 2025

ABSTRACT

Recently, clear orthodontic aligners have gained attention as devices that may improve oral health outcomes, providing faster treatment and greater comfort compared to conventional fixed braces. This review aimed to systematically assess home-care strategies for maintaining oral hygiene and cleaning aligners during orthodontic therapy. A comprehensive search was conducted across four databases: PubMed, Cochrane Library, Web of Science, and Scopus. The review, registered in PROSPERO as CRD 42024562215, followed PRISMA 2020 guidelines. Eligible studies included prospective trials, randomized controlled trials (RCTs), controlled clinical trials, and in vivo or ex vivo investigations. Studies had to compare invisible orthodontics with fixed appliances or examine home oral hygiene and aligner disinfection methods. Risk of bias was evaluated using RoB-2 for RCTs and randomized crossover trials and ROBINS-I for observational studies.
Eleven studies met inclusion criteria: four RCTs, four crossover studies, and three cross-sectional observational studies. Seven focused on patients undergoing orthodontic treatment, while four specifically investigated aligner hygiene. Cleaning effectiveness was assessed by measuring remaining biofilm on thermoplastic surfaces. Overall, the certainty of evidence was low, highlighting the need for more rigorous research. Combining chemical and mechanical cleaning approaches appears most effective for aligner care. These findings emphasize the importance of personalized home oral hygiene routines for patients undergoing treatment with clear aligners.

Keywords: Biofilm, Removable orthodontic devices, Clear aligners, Oral hygiene, Cleaning protocols

How to Cite This Article: Bona C, Camacho-Alonso F, Vaca A, Llorente-Alonso M. Oral Biofilm Control in Patients Using Orthodontic Aligners: Evidence from a Systematic Review. Asian J Periodont Orthodont. 2025;5:33-42. https://doi.org/10.51847/siIhUaqfip

Introduction

Orthodontic therapy using clear aligners has become increasingly popular in recent years. Its adoption is linked to advantages such as shorter treatment duration and greater patient comfort. The growing demand for aligners reflects society's increasing focus on aesthetics and facial harmony, which contemporary dental treatments are designed to accommodate. Simultaneously, patients seek orthodontic solutions that integrate both functional and clinical objectives with cosmetic outcomes [1].

Recent studies [2] indicate that aesthetic concerns drive orthodontic treatment in approximately 70% of patients, highlighting the preference for aligners over traditional fixed appliances due to their superior appearance and the relative ease of maintaining oral hygiene after aligner removal. Despite these benefits, the impact of aligners on soft tissue health remains not fully understood. Specific home oral hygiene practices are crucial because the aligner material is micro-rough and remains in contact with teeth for nearly the entire day, creating conditions for plaque accumulation if cleaning and disinfection are insufficient [3].

© 2025 Asian Journal of Periodontics and Orthodontics

Moreover, aligners reduce the natural protective mechanisms of the oral cavity, such as the mechanical cleansing provided by the tongue and lips, and the buffering effect of saliva [4].

Orthodontic appliances act as foreign surfaces that favor biofilm development [5], with colonization patterns influenced by surface morphology, roughness, and the availability of oxygen or nutrients, which in turn affect bacterial interactions and biofilm structure [6]. In the context of orthodontic treatment, long-term supragingival plaque predominantly consists of facultative anaerobic bacteria with cariogenic potential (e.g., *Streptococcus mutans*, *S. salivarius*, and *S. sobrinus*, Gram-positive), while bacteria with periodontal relevance, such as *Aggregatibacter actinomycetemcomitans* (Gram-negative), are less abundant [3].

Despite suggestions in previous studies [7] that clear aligners may help maintain periodontal health better than fixed braces by limiting plaque retention and avoiding bacterial adherence to metal surfaces, comprehensive data on the oral microbiome in aligner users remain limited. In patients with conventional fixed appliances, anaerobic bacterial communities are mainly detected on enamel surfaces [8], including Flavobacteriaceae (e.g., *Capnocytophaga sputigena*, Gram-negative, associated with periodontal inflammation), Prevotellaceae (e.g., *Prevotella intermedia*, anaerobic, involved in protein and carbohydrate metabolism and often linked to periodontal disease), and Saccharimonadaceae (related to oral mucosal infections).

By contrast, aligner wearers show higher abundance of Burkholderiaceae (e.g., *Burkholderia cepacia*, Gram-negative, typically not found in the oral cavity but present in cystic fibrosis lung infections) [8]. Changes in the oral environment induced by aligners can alter bacterial composition or activity [9], leading to differences in amino acid metabolism compared with the normal oral microbiome. Studies report that salivary and tooth-surface microbial communities change within 12 hours after aligner placement [10].

Thermoplastic aligners fully cover teeth and are removable, which creates a distinct environment for bacterial growth compared to exposed surfaces in fixed orthodontics [11]. The microbiome shift, including increases in families linked to periodontal pathogens, emphasizes the importance of personalized and intensified oral hygiene strategies for both removable and fixed orthodontic devices [12].

Current systematic reviews [4] focus primarily on aligner cleaning techniques, such as mechanical methods (toothbrushing, vibration), chemical approaches (chlorhexidine, anionic/cationic detergents, effervescent tablets), or combinations of both. However, broader protocols for daily home oral care throughout treatment are still lacking. Methods that work for fixed appliances may not fully apply to removable aligners, particularly regarding biofilm and plaque management.

Based on these considerations, this review aims to investigate the most effective home oral hygiene strategies for preserving periodontal and dental health during clear aligner therapy.

Materials and Methods

This review adhered to the PRISMA 2020 guidelines and is registered in PROSPERO under the identification number CRD 42024562215.

The PICOS framework was defined as follows:

- *Participants:* healthy individuals undergoing treatment with clear, removable orthodontic appliances;
- *Interventions:* home-based oral hygiene routines;
- *Comparators:* either negative controls or placebo conditions;
- *Outcomes:* evaluation of bacterial biofilm formation;
- *Study design:* prospective investigations, randomized controlled trials (RCTs), and controlled clinical trials published in English within the last 20 years, which assessed the impact of at-home hygiene strategies on either patients or their aligners.

Eligibility criteria and central questions

Included studies were limited to prospective studies, RCTs, controlled clinical trials, and in vivo or ex vivo studies. They needed to investigate either:

- treatment using clear aligners;
- comparisons between clear aligners and traditional fixed appliances;
- patient-managed oral hygiene protocols; or
- aligner cleaning protocols performed at home.

Studies were excluded if they were systematic reviews, case reports or series, animal studies, in vitro experiments, surgical orthodontic interventions, or focused solely on fixed appliance protocols. Research not evaluating at-home hygiene strategies was also omitted.

This review focused on two primary questions:

1. Which home care practices are most effective for maintaining oral hygiene while using clear aligners?

2. How should clear aligners be cleaned to minimize bacterial biofilm formation during treatment?

Search strategy

A comprehensive search was conducted in PubMed, Cochrane Library, Scopus, and Web of Science to identify relevant studies. A single search string was applied to PubMed, Cochrane, and Web of Science, while a custom query was used for Scopus. Keywords included variations of clear aligners, removable appliances, oral hygiene, biofilm, and cleaning methods, combined with Boolean operators (AND, OR, NOT) to capture all relevant literature on aligner cleaning and home oral hygiene protocols.

Screening and selection

Two reviewers (AP, SG) independently screened the search results. Duplicates, inaccessible studies, non-English articles, and those published before 2003 were removed. Titles and abstracts were then assessed, and irrelevant studies were excluded. Full texts of potentially relevant articles were examined for inclusion, with disagreements resolved by a third reviewer (AS). Data on study details, population, interventions, controls, and outcomes were extracted independently by AP and SG and managed using Review Manager (RevMan) Version 5.4 (Copenhagen: Nordic Cochrane Center, 2003).

Assessment of risk of bias

The methodological rigor of each included study was evaluated independently by AP and SG, with disagreements resolved by the intervention of a third reviewer (AS). The risk of bias assessment tools were chosen according to the study design. Randomized controlled trials (RCTs) and randomized crossover studies were evaluated using the Cochrane RoB-2 tool [13], whereas cross-sectional observational studies were assessed with the Cochrane ROBINS-I tool [14].

Results and Discussion

Figure 1 illustrates the full selection pathway, starting from the database searches to the final set of studies included in this review.

A total of 502 records were retrieved from the four electronic databases (PubMed: 116; Cochrane Library: 188; Web of Science: 164; Scopus: 34), with two additional articles identified through manual reference searches. Before screening, 273 items were removed due to duplication (103), inaccessibility (169), or publication in languages other than English (1). Titles and abstracts of the remaining 231 articles were then examined, resulting in the exclusion of 220 studies. Specific reasons included: 5 studies outside the eligible publication period, 18 with ineligible study designs, 134 irrelevant to the topic, 31 not addressing invisible orthodontic treatment, 1 including patients with pathologies, and 31 failing to report home hygiene protocols (22 not addressing patient oral hygiene, 9 not describing aligner cleaning).

Following full-text assessment, nine studies were deemed suitable for inclusion. Of these, four originated from PubMed [15–18], one from the Cochrane Library [19], and four from the Web of Science [20–23]. No studies from Scopus met the eligibility criteria. These nine studies were combined with the two identified manually, resulting in a final total of 11 studies included in this systematic review.

Study characteristics

The main attributes of the included studies are presented in **Table 1**. Among the 11 studies, 4 were randomized controlled trials (RCTs), 4 were crossover studies, and 3 were cross-sectional observational studies. All publications were dated between 2013 and 2022. Seven studies involved participants with invisible orthodontic devices or mixed groups comparing invisible and fixed appliances, while four focused exclusively on aligners, with participants using between 3 and 12 aligner sets.

Seven studies assessed the efficiency of home oral hygiene measures in patients with invisible orthodontics, comparing outcomes with patients using fixed appliances, with or without control groups. The remaining four studies explored various cleaning and disinfection approaches for aligner templates. Clinical outcomes were mainly evaluated using periodontal indices and microbiological assessments, with plaque index (PI), gingival index (GI), bleeding on probing (BoP), and probing pocket depth (PPD) being the most commonly reported measures. For the non-clinical studies, microbiological analyses included scanning electron microscopy (SEM), photodensitometry, and ATP measurements via a bioluminometer.

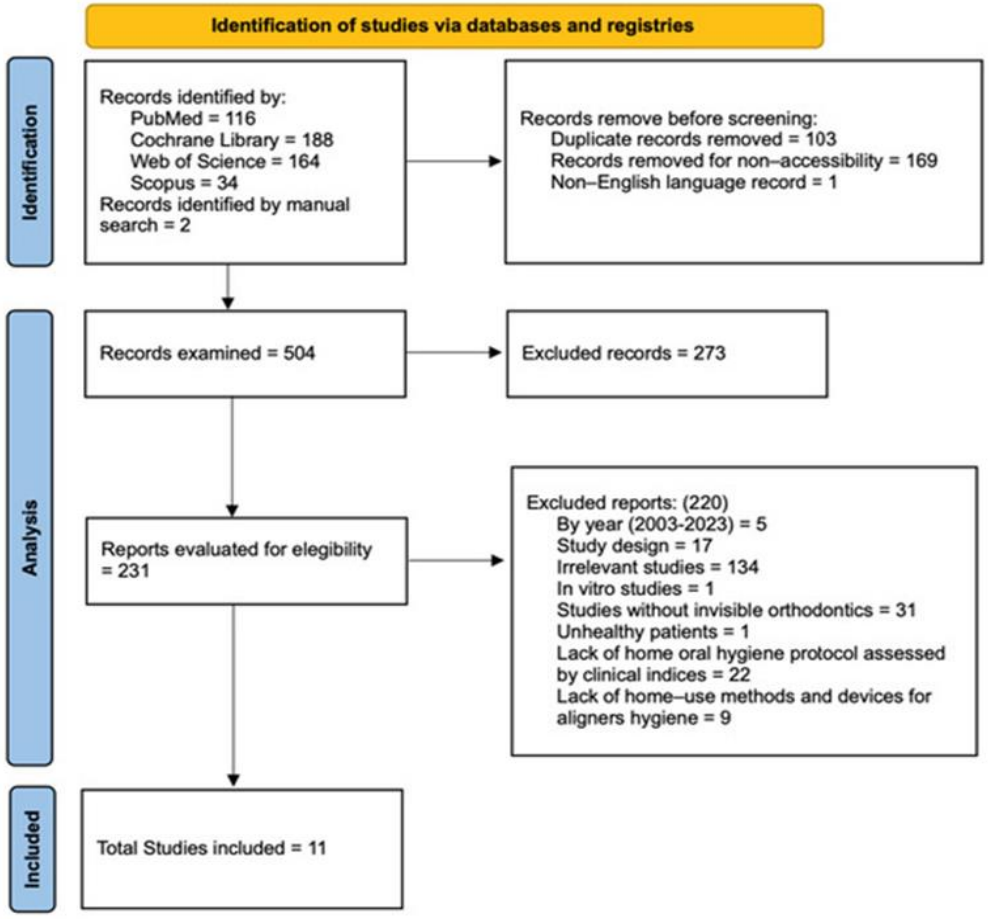


Figure 1. Selection of studies. Flow diagram following the PRISMA 2020 guidelines [24].

Table 1. Summary of studies incorporated in the review.

Author	Year	Publication	Research Design	Participants	Procedure	Assessed Metrics
Azaripour *et al*. [22]	2015	BMC Oral Health	Snapshot study	100 people; 50 with clear aligners, 50 with conventional braces	Daily oral care routine	Gum condition score, bleeding in sulcus, plaque buildup index
Caccianiga *et al*. [23]	2022	Healthcare	Observational snapshot	50 people; 25 with clear aligners, 25 with fixed braces	Routine dental hygiene	Bacterial profile analysis (harmful vs. harmless), plaque examined via SEM
Chhibber *et al*. [19]	2018	American Journal of Orthodontics and Dentofacial Orthopedics	Controlled randomized trial	61 people; 24 with clear aligners, 37 with fixed braces (17 self-ligating, 20 elastomeric)	Home dental care practices	Plaque level, gum irritation score, bleeding papillae index
Levrini *et al*. [25]	2013	Cumhuriyet Dent J	Controlled randomized trial	30 people; 10 with clear aligners, 10 with fixed braces, 10 without braces (control)	Personal oral hygiene regimen	Plaque level, gum pocket depth, probing-induced bleeding, bacterial detection via real-time PCR
Levrini *et al*. [26]	2015	European Journal of Dentistry	Controlled randomized trial	77 people; 32 with clear aligners, 35 with fixed braces, 10 without braces (control)	At-home dental care	Plaque level, gum pocket depth, probing-induced bleeding, bacterial detection via real-time PCR
Levrini *et al*. [18]	2015	Clinical, Cosmetic and Investigational Dentistry	Crossover trial	36 aligners (3 per 12 individuals)	Aligner cleaning method	Plaque volume assessed via SEM

Levrini *et al*. [16]	2016	International Journal of Dentistry	Crossover trial	36 aligners (3 per 12 individuals)	Aligner maintenance protocol	Bacterial levels measured by ATP with bioluminometer, reported in RLUs
Lombardo *et al*. [17]	2017	Progress in Orthodontics	Crossover trial	45 aligners (9 per 5 individuals)	Aligner hygiene routine	Biofilm presence via SEM, evaluated on Grey scale
Sfondrini *et al*. [21]	2021	Applied Sciences	Controlled randomized trial	40 people; 20 with clear aligners, 20 without braces (control)	Daily oral hygiene practices	Plaque level, probing-induced bleeding, gum pocket depth, bacterial analysis via real-time PCR
Shpack *et al*. [15]	2014	Angle Orthodontist	Crossover trial	132 aligners (12 per 11 individuals)	Aligner cleaning routine	Biofilm adhesion measured with photodensitometer
Zhao *et al*. [20]	2020	Oral Diseases	Snapshot study	25 people with clear aligners	Home-based oral care	Plaque level, gum pocket depth, probing-induced bleeding

Key findings of the review

The results of the included studies were categorized according to the type of protocol investigated: aligner cleaning and disinfection outcomes are presented in **Table 2**, while findings on patients' at-home oral hygiene practices are summarized in **Table 3**.

In the first category, all four studies assessed the effectiveness of hygiene protocols on bacterial adhesion to aligners. Two studies [16, 26] employed scanning electron microscopy (SEM) and ATP bioluminometry, reporting that the most effective cleaning method combined brushing with a soluble tablet containing sodium carbonate and sodium sulfate. The remaining two studies [15, 17] observed statistically significant improvements ($p < 0.05$) when aligners were immersed in either an ultrasonic bath with a cationic detergent or chlorhexidine. Collectively, the findings indicate that coupling mechanical cleaning with chemical or device-assisted methods enhances overall aligner hygiene.

For the second category, focusing on home oral hygiene, six out of seven studies [18, 20–23, 25] reported a significant increase in microbial colonization among patients with fixed appliances compared to those using invisible aligners ($p < 0.05$). Various periodontal and plaque indices were evaluated: gingival index (GI) rose in fixed orthodontic patients ($p = 0.001$) [22] but decreased by 86% in aligner users ($p = 0.015$) [19]. Probing depth (PD), bleeding on probing (BOP), and plaque index (PI) decreased in favor of aligner users in two studies [18, 25], whereas two other studies [20, 21] did not observe significant differences across these indices.

Table 2. Results and findings from studies evaluating aligner cleaning methods.

Researcher (Year)	Cleaning Protocol	Measured Outcome	Findings
Levrini *et al*. (2015) [26]	2 weeks: rinse aligners with cold water for 15 s twice daily (control); 2 weeks: soak aligners in cold water with a dissolvable tablet (sodium carbonate and sulfate) for 30 min, then brush with soft toothbrush and medium-abrasive toothpaste (RDA < 150) for 30 s; 2 weeks: brush aligners with soft toothbrush and medium-abrasive toothpaste (RDA < 150) for 30 s.	Plaque quantity assessed via scanning electron microscopy (SEM).	Group 3 (brushing only) outperformed the control (Group 1) on outer surfaces. Group 2 (tablet soaking) showed the best results. No differences were observed on inner surfaces. Bacterial contamination was mostly organic, with rare inorganic tartar. Only one type of spherical bacteria was detected.
Levrini *et al*. (2016) [16]	2 weeks: rinse aligners with cold water for 15 s after removal (control); 2 weeks: brush aligners with soft toothbrush and low-abrasive toothpaste (RDA < 100) for 30 s; 2 weeks: soak aligners in cold water with dissolvable tablet (sodium carbonate and sulfate) for 20 min, then brush with soft toothbrush and low-abrasive toothpaste (RDA < 100) for 30 s.	Bacterial load measured by ATP levels using a bioluminometer, reported in RLUs.	Mean bacterial loads: Group 1 = 583 RLU, Group 2 = 188 RLU, Group 3 = 71 RLU. Median values: Group 1 = 518 RLU, Group 2 = 145 RLU, Group 3 = 64 RLU. Group 3 (tablet soaking and brushing) had the lowest bacterial load, significantly lower than Group 1 ($p = 0.0003$).

Lombardo *et al*. (2017) [17]	2 weeks each: rinse with water; sonic bath with water; ultrasonic bath with water; water bath with anionic detergent; sonic bath with anionic detergent; ultrasonic bath with anionic detergent; water bath with cationic detergent; sonic bath with cationic detergent; ultrasonic bath with cationic detergent. Each method lasted 5 min, twice daily.	Biofilm presence evaluated via SEM, measured on a Grey scale.	Method 1 (water rinse) was the least effective, while Method 9 (ultrasonic bath with cationic detergent) was the most effective ($p < 0.05$). All methods except Method 1 reduced biofilm effectively.
Shpack *et al*. (2014) [15]	28 days: brush teeth and aligners with 1400 ppm fluoride toothpaste (control); 70 days: brush aligners, then soak in chlorhexidine mouthwash for 15 min nightly, rinse before reinsertion; 70 days: soak aligners in vibrating bath with cleaning crystal solution for 15 min nightly, rinse before reinsertion. Aligners were stained with 1% gentian violet for 5 min post-protocol.	Biofilm adhesion measured by photodensitometer.	Protocols 2 (chlorhexidine) and 3 (vibrating bath) significantly reduced biofilm ($p < 0.001$), with 16% and 50% reductions, respectively. Protocol 1 (brushing only) showed higher plaque buildup on posterior palatine and incisal edge areas.

Table 3. Findings and outcomes of the included studies regarding patients' at-home oral hygiene practices.

Researcher (Year)	Cleaning Protocol	Measured Outcomes	Findings
Azaripour *et al*. (2015) [22]	Three daily uses: toothbrush, dental floss, pipe cleaner	Gingival index (GI), sulcus bleeding index (SBI), approximal plaque index (API) via plaque detector tablet	Both fixed orthodontics and aligner groups showed increased SBI and GI from treatment start to follow-up. Fixed orthodontics had significantly worse outcomes (SBI: $p < 0.001$; GI: $p = 0.001$), indicating poorer gingival health compared to clear aligners.
Caccianiga *et al*. (2022) [23]	Fixed orthodontics: orthodontic-head toothbrush, single-tufted toothbrush, regular toothbrush. Invisible orthodontics: soft-bristled toothbrush, flossing. For pathogenic flora at T1 (twice daily): sonic toothbrush, regular toothbrush, water flosser	Subgingival plaque quality via SEM, distinguishing pathogenic vs. non-pathogenic flora	At T1 (3 months), 10/25 fixed orthodontics and 3/25 aligner patients had pathogenic flora. After modified hygiene protocol, no pathogenic flora was detected at T2 (6 months). Fixed orthodontics showed a significant correlation with pathogenic flora ($p = 0.024$).
Chhibber *et al*. (2017) [19]	General oral hygiene: toothpaste, sonic toothbrush, regular toothbrush, dental floss	Plaque index (PI), gingival index (GI), papillary bleeding index (PBI)	No significant differences in PI, GI, or PBI among aligners, self-ligating, and elastomeric fixed braces after 18 months (T2). At 9 months (T1), aligners showed 86% lower gingival inflammation ($p = 0.015$) and 90% lower papillary bleeding ($p = 0.012$).
Levrini *et al*. (2013) [25]	Three daily practices: orthodontic-head toothbrush (Bass technique, 2 min), flossing	Plaque index, pocket probing depth (PD), bleeding on probing (BOP), biofilm presence via real-time PCR	Aligner patients had reduced pocket depth ($p = 0.002$) and BOP ($p < 0.001$) at T2 (3 months) vs. T1 (1 month). Fixed orthodontics correlated with higher PI ($p < 0.001$), BOP ($p < 0.001$), lower hygiene compliance ($p < 0.001$), and increased biofilm ($p < 0.005$), indicating less plaque and periodontal risk with aligners.
Levrini *et al*. (2015) [18]	Three daily practices: orthodontic-head toothbrush (Bass technique, 2 min), flossing	Plaque index, pocket probing depth, bleeding on probing, biofilm presence via real-time PCR	Aligners showed significantly better outcomes for PI, PD, and BOP ($p < 0.05$) compared to fixed orthodontics. Fixed appliances had higher biofilm levels ($p < 0.05$), with worse periodontal indices at T2 compared to T0 and T1.

Sfondrini *et al*. (2021) [21]	Three daily: electric toothbrush (2 min); once daily: floss	Plaque index, probing pocket depth, bleeding on probing, bacterial flora analysis via real-time PCR	No significant changes in PI, PPD, or BOP in test or control groups. Bacterial species distribution remained stable. Total bacterial count increased significantly ($p < 0.05$) from T0 (14 days post-professional hygiene) to T1 (2 months) in both groups.
Zhao *et al*. (2020) [20]	After meals/snacks: toothbrush (Bass technique), flossing	Plaque index, pocket probing depth, bleeding on probing	PI significantly decreased ($p < 0.05$) after 6 months. BOP and PPD showed no significant changes. Brushing frequency increased significantly ($p < 0.05$) during treatment.

Risk of bias

Figure 2 displays the bias assessment results for the randomized crossover clinical trials included in this review. The modified Rob-2 instrument, specifically designed for this study type, was applied. Overall, the trials showed a high risk of bias, mainly due to issues in the randomization process domain present in all studies. Consequently, the confidence in the evidence derived from these trials remains limited.

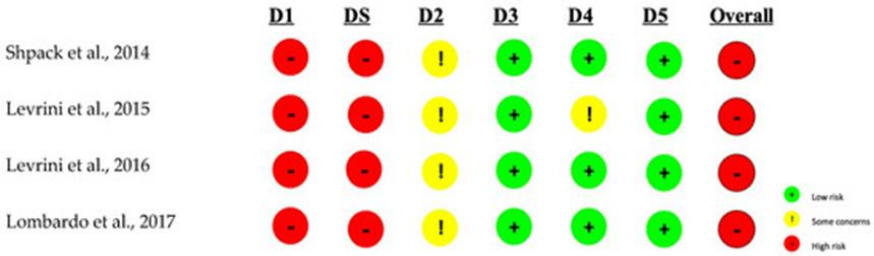


Figure 2. Risk of Bias Evaluation in Modified Rob-2 Crossover Randomized Trials. Assessed domains: D1: bias from the randomization procedure; DS: bias associated with period effects or carryover; D2: bias due to deviations from the intended interventions; D3: bias caused by missing outcome information; D4: bias in outcome measurement; D5: bias from selective reporting of outcomes [15, 16, 17, 26].

For the randomized controlled trials (RCTs), the Rob-2 tool was similarly employed. The overall bias assessment ranged from moderate to high across studies **(Figure 3)**. Even though several domains indicated low risk, the study by Levrini *et al*. [25] was rated high because of problems in randomization, while the studies by Levrini *et al*. [26], Chhibber *et al*. [19], and Sfondrini *et al*. [21] were considered moderate risk. The moderate classification largely arose from domain 2 ("some concerns"), reflecting the unavoidable awareness of participants and investigators regarding treatment allocation. Blinding was not feasible in these trials due to the visible nature of the orthodontic interventions.



Figure 3. Risk of Bias Evaluation in Rob-2 Randomized Controlled Trials. Domains assessed: D1: Bias originating from randomization; D2: Bias due to deviations from intended interventions; D3: Bias caused by incomplete outcome data; D4: Bias in outcome measurement; D5: Bias from selective reporting of results [18, 19, 21, 25].

The assessment indicates that the evidence from these trials should be interpreted with caution, reflecting inherent limitations related to the intervention rather than solely the methodological rigor of the included studies.

For the cross-sectional observational studies, the Cochrane ROBINS-I tool was applied. Most domains

demonstrated a low risk of bias, except for outcome measurement, which was moderate in the studies by Azaripour *et al*. [22] and Zhao *et al*. [20]. This moderate rating was attributable to examiners being aware of the participants' orthodontic treatment type. A double-blind design could not be implemented, as outcome evaluation required the assessment of periodontal indices by researchers who could not be blinded to whether participants had fixed or removable aligners. Accordingly, the overall risk of bias for these studies was considered low to moderate. Similar to the RCTs, these results should be interpreted with consideration of the intervention's inherent constraints when evaluating the evidence quality.

The application of clear aligners has grown substantially in orthodontic practice, particularly for adults, while fixed appliances remain more common in younger patients. Aligners are favored because they combine aesthetic appeal with simplified oral hygiene. Evidence consistently shows that fixed braces are prone to plaque accumulation due to cleaning challenges [12, 22, 27–30], whereas removable aligners allow hygiene routines comparable to individuals without orthodontic appliances.

Clear aligners are also considered suitable for patients with existing periodontal issues or higher susceptibility to periodontitis. Research suggests that these patients experience no elevated risk of gingivitis or periodontitis and may even benefit from improved periodontal conditions [27, 28, 31, 32]. This improvement is largely due to the easier removal of biofilm, which is the primary trigger for gingival inflammation and, if left unmanaged, for periodontitis [33].

Nonetheless, inadequate hygiene of the mouth and the aligners can lead to bacterial buildup, with biofilm formation detectable within two weeks—the typical interval before aligner replacement [15]. The plastic surface of aligners, including micro-abrasions and irregularities, further facilitates microbial adhesion [3]. Biofilm can spread across both dental and aligner surfaces, especially because these surfaces are not naturally exfoliating [4].

The review identified effective strategies to reduce bacterial colonization. Levrini *et al*. [16, 26] evaluated three disinfection methods using SEM and ATP bioluminometry and concluded that immersing aligners in a water-soluble tablet containing sodium carbonate and sodium sulfate, combined with brushing using a soft toothbrush and moderately abrasive toothpaste, provided the best results. These findings highlight the advantage of combining chemical and mechanical actions rather than relying on a single approach. While not completely eliminating bacterial residues, this combination is considerably more effective than simple water rinsing.

Lombardo *et al*. [17] tested nine cleaning techniques over two weeks. Simple rinsing under running water was least effective. Other combinations of sonic or ultrasonic baths with cationic and anionic detergents all reduced bacterial load, but the combination of an ultrasonic bath and cationic detergent was most effective. Using a vibrating bath with cleansing crystals yielded three times better results than chlorhexidine immersion alone [15]. These results emphasize the necessity of mechanical action, such as brushing or ultrasonic devices, since soft plaque can start turning into semi-calcified deposits after two weeks of aligner use [15–17].

Regarding at-home oral hygiene, one study compared fixed and removable orthodontics using toothbrushes, floss, and interdental brushes [19]. No significant differences in periodontal outcomes were observed between groups or over time. Another study focusing solely on aligners [21] found that electric brushing three times daily with flossing once daily did not significantly improve oral hygiene. Similarly, manual brushing using the Bass technique plus flossing after meals showed only a decrease in plaque index after six months, without changes in other periodontal indices [20].

In contrast, several studies demonstrated improved periodontal health in aligner users following specific hygiene protocols. Azaripour *et al*. [22] reported notable reductions in gingival inflammation in aligner patients using a manual toothbrush, floss, and pipe cleaner three times daily compared to fixed appliance users. Levrini *et al*. [16, 26] found better periodontal indices and higher patient adherence when using a toothbrush with an orthodontic head, the Bass technique for 2 minutes, and floss. Caccianiga *et al*. [23] analyzed pathogenic oral flora and observed fewer harmful bacteria in aligner users employing a soft toothbrush and floss versus fixed appliance users after three months. With a modified protocol involving a sonic toothbrush, water flosser, and interdental brush, all previously affected subjects no longer exhibited pathogenic flora.

The review's limitations include low overall evidence quality due to high risk of bias, particularly related to randomization. Among hygiene-focused studies, some were RCTs while others were observational. Randomized trials mostly carried moderate to high bias risk, whereas cross-sectional studies had low to moderate risk. The diversity of at-home hygiene protocols highlights the importance of personalized

methods for both dental care and aligner maintenance. Future research should develop protocols tailored to individual abilities, adherence levels, and the combined use of chemical and mechanical interventions, while also evaluating long-term outcomes beyond the treatment period.

Conclusion

Given the heterogeneity of the studies included in this systematic review, it is not possible to definitively identify a single most effective home oral hygiene protocol during treatment with invisible orthodontics. Methodological limitations, particularly the absence of proper randomization and the impracticality of applying blinded or double-blind designs, resulted in a low level of certainty in the evidence, indicating that further research is required. Clinically, regarding strategies to maintain aligner cleanliness, although mechanical methods alone appear slightly more effective than chemical approaches, the combination of mechanical and chemical interventions produced the best results. This finding underscores the importance for patients to adopt specific combined protocols for home aligner hygiene.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Shaw WC, O'Brien KD, Richmond S. Quality control in orthodontics: factors influencing the receipt of orthodontic treatment. Br Dent J. 1991;170(2):66–8.
2. Saccomanno S, Saran S, Laganà D, Mastrapasqua RF, Grippaudo C. Motivation, perception, and behavior of the adult orthodontic patient: a survey analysis. Biomed Res Int. 2022;2022(1):2754051.
3. Low B, Wilson L, 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.
4. Charavet C, Gourdain Z, Graveline L, Lupi L. Cleaning and disinfection protocols for clear orthodontic aligners: a systematic review. Healthcare (Basel). 2022;10(2):340.
5. Bowden GH, Li YH. Nutritional influences on biofilm development. Adv Dent Res. 1997;11(1):81–99.
6. Stodley P, Dodds I, Boyle JD, Lappin-Scott HM. Influence of hydrodynamics and nutrients on biofilm structure. J Appl Microbiol. 1998;85(S1):19–28.
7. Ahn SJ, Lim BS, Lee YK, Nahm DS. Quantitative determination of adhesion patterns of cariogenic streptococci to various orthodontic adhesives. Angle Orthod. 2006;76(5):869–75.
8. Zheng J, Wang X, Zhang T, Jiang J, Wu J. Comparative characterization of supragingival plaque microbiomes in malocclusion adult female patients undergoing orthodontic treatment with removable aligners or fixed appliances: a descriptive cross-sectional study. Front Cell Infect Microbiol. 2024;14:1350181.
9. Nascimento MM, Alvarez AJ, Huang X, Browngardt C, Jenkins R, Sinhoreti MC, et al. Metabolic profile of supragingival plaque exposed to arginine and fluoride. J Dent Res. 2019;98(11):1245–52.
10. Yan D, Liu Y, Che X, Mi S, Jiao Y, Guo L, et al. Changes in the microbiome of the inner surface of clear aligners after different usage periods. Curr Microbiol. 2021;78(2):566–75.
11. Lucchese A, Nocini R, Lo Giudice A, Asperio P, Guglietta F, Carenzi L, et al. Oral and throat microbiological changes after orthodontic debonding. New Microbiol. 2022;45(4):278–83.
12. Abbate GM, Caria MP, Montanari P, Mannu C, Orru G, Caprioglio A, et al. Periodontal health in teenagers treated with removable aligners and fixed orthodontic appliances. J Orofac Orthop. 2015;76(3):240–50.
13. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ. 2019;366:l4898.
14. Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: A tool for assessing risk of bias in non-randomised studies of interventions. BMJ. 2016;355:i4919.
15. Shpack N, Greenstein RB, Gazit D, Sarig R, Dan Vardimon A. Efficacy of three hygienic protocols in reducing biofilm adherence to removable thermoplastic appliance. Angle Orthod. 2014;84(1):161–70.
16. Levrini L, Mangano A, Margherini S, Tenconi C, Vigetti D, Muollo R, et al. ATP bioluminometers analysis on the surfaces of removable orthodontic

aligners after the use of different cleaning methods. Int J Dent. 2016;2016(1):5926941.
17. 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):26.
18. 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.
19. Chhibber A, Agarwal S, Yadav S, Kuo C, Upadhyay M. Which orthodontic appliance is best for oral hygiene? A randomized clinical trial. Am J Orthod Dentofacial Orthop. 2018;153(2):175–83.
20. 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.
21. Sfondrini MF, Butera A, Di Michele P. Microbiological changes during orthodontic aligner therapy: a prospective clinical trial. Appl Sci. 2021;11(15):6758.
22. Azaripour A, Weusmann J, Mahmoodi B, Peppas D, Gerhold-Ay A, Van Noorden CJF, et al. Braces versus Invisalign: gingival parameters and patients' satisfaction during treatment: a cross-sectional study. BMC Oral Health. 2015;15(1):69.
23. Caccianiga P, Nota A, Tecco S, Ceraulo S, Caccianiga G. Efficacy of home oral-hygiene protocols during orthodontic treatment with multibrackets and clear aligners: microbiological analysis with phase-contrast microscope. Healthcare (Basel). 2022;10(11):2255.
24. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.
25. Levrini L, Abbate GM. Assessment of the periodontal health status in patients undergoing orthodontic treatment with fixed or removable appliances: a microbiological and preliminary clinical study. Cumhur Dent J. 2013;16(4):296–307.
26. 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-month clinical and microbiological evaluation. Eur J Dent. 2015;9(03):404–10.
27. Miethke RR, Vogt S. A comparison of the periodontal health of patients during treatment with the Invisalign system and with fixed orthodontic appliances. J Orofac Orthop. 2005;66(3):219–29.
28. Miethke R, Vogt S. A comparison of the periodontal health of patients during treatment with the Invisalign system and with fixed lingual appliances. J Orofac Orthop. 2007;68(3):223–31.
29. Cantekin K, Celikoglu M, Karadas M, Yildirim H, Erdem A. Effects of orthodontic treatment with fixed appliances on oral health status: a comprehensive study. J Dent Sci. 2011;6(4):235–8.
30. Pardo A, Baccini F, De Manzoni R. Removal of bacterial biofilm in patients undergoing fixed orthodontic treatment: a literature review. J Appl Cosmetol. 2023;41(1):1–12.
31. Faccioni P, Butera A, Bazzanella S. 3D evaluation of upper airway morphological changes in growing patients with class II malocclusion using Sander bite jumping appliance. Appl Sci. 2023;13(6):3908.
32. Faccioni P, Sacchetto L, Sinigaglia S. An improvement of upper airway flow in patients treated with rapid maxillary expansion: a cone beam computed tomography study. J Appl Cosmetol. 2023;41(1):99–106.
33. Löe H. Physiology of the gingival pocket. Acad Rev Calif Acad Periodontol. 1965;13(1):6–14.