

Microbial Adhesion and Biofilm Formation on Clear Aligners: A Literature Review

VP SHRAVANI¹, RAVINDRA KUMAR JAIN²

ABSTRACT

Oral cavity is a host for a variety of microorganisms, these microbes tend to adhere to retentive areas in the oral cavity such as deep occlusal grooves on posterior teeth, interdental areas and form biofilm. This leads to the development of dental caries and gingivitis. Patients undergoing fixed appliance orthodontic treatment are more prone for enamel demineralisation caused by adherence of microbial colonies. Clear aligners, which are made of clear thermoplastic material are commonly used in clinical practice recently. Clear aligners are a series of plastic trays which have to be worn for 22 hours a day for effective tooth movement and have to be changed for every two weeks. These aligners cover the tooth completely, making it difficult for the antibacterial effect of saliva to act on the tooth surfaces. Hence, the present review aimed to assess the microbial adhesion and biofilm formation in patients undergoing clear aligner therapy and the effectiveness of various methods to clean aligners.

Keywords: Bacterial adhesion, Dental plaque, Disinfection, Orthodontic appliances

INTRODUCTION

Oral biofilm, also known as dental plaque is an extensive network of bacteria encased in an extracellular substrate generated by microorganisms that mostly cling to gingival epithelial cells or tooth surfaces. It serves as acellular protein-based coating that covers the enamel surface and serves as a site for bacterial attachment, which is regarded as a prerequisite for the bacterial colonisation of the tooth and other oral surfaces [1].

Most common complication of orthodontic appliances are enamel demineralisation and gingivitis [2]. Orthodontic appliances alter and increase the microbiota because they offer areas for bacteria to congregate and make cleaning difficult [3]. Maintaining oral hygiene during orthodontic treatment is critical as orthodontic appliances promote food accumulation and plaque retention. This increases the risk of enamel demineralisation and gingival inflammation [4]. White Spot Lesions (WSL) are consequences of enamel demineralisation caused by acid producing caries-associated bacteria. WSL appear as chalky white opaque enamel. It is an unfavourable complication of orthodontic treatment [5]. The risk of caries is linked to oral microenvironment, and the later plays a pivotal role in the genesis and progression of caries [6].

Orthodontic fixed appliances tend to alter the oral microbiota, with increase in the levels of *Streptococcus mutans* and *Lactobacillus* species, noted especially after bonding [7]. These appliances favour biofilm formation [8,9]. A study by Yan D et al., observed higher levels of *P. gingivalis*, *P. intermedia/Prevotella nigrescens*, *T. forsythia*, and *Fusobacterium* spp. in orthodontic patients three months after orthodontic treatment [9,10]. Similarly, Pan S et al., observed an increase in several periodontopathic bacteria three months after the start of orthodontic therapy [11].

Invisible orthodontic treatment, being preferred by many patients involves the use of clear aligners [12]. These are transparent polymer materials which can be thermoformed or directly printed and are available as a series of trays. These trays are to be changed every two weeks for the predicted tooth movement to take place. Compared with the traditional orthodontic appliances, clear aligners cover all teeth surfaces, which hinder the antibacterial, remineralising potential, and cleansing ability of saliva [13]. Drawbacks of these clear aligners include discolouration, appliance loss, biofilm build-

up, the emission of potentially cytotoxic or oestrogenic substances, and the necessity for patient participation [14].

Understanding the biofilm formation and microbial adhesion on tooth surfaces is essential for maintaining good oral health and preventing enamel decalcification. And it is also essential to encourage patients to keep their aligners clean to prevent the development of WSL. Hence, the present review aimed to assess the existing literature evidence on the initial microbial adhesion and biofilm formation on clear aligner materials.

A thorough search was run in PubMed, Scopus and Web of science along with the use of google scholar search engine. Articles published in English and dated from 2010-2024 were included.

The following search strategy was used: (Microbial adhesion OR microbial colonisation) AND (orthodontic treatment OR orthodontic therapy OR clear aligners OR clear aligner therapy).

Eligibility criteria: Studies which have quantified biofilm formation and microbial adhesion in patients treated using clear aligner therapy have been included. Studies that include methods of cleaning or disinfecting aligners have been included in the current review.

Plaque and oral microbiota: There are over 700 distinct types of microorganisms, including both anaerobic and aerobic bacteria [15]. The gram-positive and gram-negative organisms in the oral cavity are represented in [Table/Fig-1] [16]. Supra gingival plaque is considered to be a causative agent for enamel demineralisation, with the presence of the above bacterial species [17].

DISCUSSION

Bacterial Adhesion

Bacterial adhesion depends on various factors such as duration of exposure, bacterial characteristics, nutrients, and bacterial density [18]. Initially bacteria attach to a surface by vanderwaals forces and electrostatic forces caused by ionic groups [19].

Streptococcus (S.) *sanguis* appears to have a preference for colonising on tooth surfaces, whereas *S.salivarius* has a marked affinity for epithelial surfaces and shows little affinity to adhere to teeth. It has been observed that plaque bacteria *S. sanguis*, *S.mitis*, and *Actinomyces* species, but not *S. mutans* or *S. salivarius* aggregate in presence of human saliva. Also, *S. sanguis* and *Actinomyces*

Gram positive organisms	Gram negative organisms
<i>Streptococcus</i>	<i>E. coli</i>
<i>Lactobacillus</i>	<i>Klebsiella</i>
<i>Staphylococcus</i>	<i>Enterobacter</i>
<i>Clostridium</i>	<i>Enterococcus</i>
<i>Bacillus</i>	<i>Citrobacter</i>
<i>Corynebacterium</i>	<i>Vibrio</i> spp.
<i>Actinomyces</i>	<i>Campylobacter</i>
<i>Propionibacterium</i>	<i>Helicobacter pylori</i>
<i>Rothia</i>	<i>Proteus</i>
<i>Eubacterium</i>	<i>Fusobacterium</i>
<i>Gemella</i>	<i>Neisseria</i>
<i>Peptostreptococcus</i>	<i>Moraxella</i>
<i>Enterococcus faecalis</i>	<i>Kingella</i>
Leuconostocaceae	<i>Actinobacillus</i>
Listeriaceae	<i>Bacteroides</i>
	<i>Porphyromonas</i>
	<i>Prevotella</i>
	<i>Selenomonas</i>
	<i>Cardiobacterium</i>
	<i>Eikenella</i>
	<i>Veillonella</i>
	<i>Leminorella</i>
	<i>Morganella</i>
	<i>Providencia</i>
	<i>Yersinia</i>
	<i>Serratia</i>

[Table/Fig-1]: Microorganisms in the oral cavity.

have been observed to adhere to saliva coated enamel, whereas lactobacilli, *S. mutans* and *S. salivarius* appear to be unaffected or demonstrate little ability to adhere to saliva coated enamel.

Straub H et al., classified bacterial adhesion into three different levels, which includes: (1) specific physical-chemical interactions; (2) Non specific interactions; and (3) surface mechanosensing [Table/Fig-2] [20-22].

Type of interactions	Key features
Specific microscopic interactions	Occur at very short distances between surface components that are stereochemically complimentary; particularly hydrogen, ionic, and may be chemical connections [20]
Non specific interactions	Interactions due to change in surface free energy. These interactions can occur over very long distances, making it impossible for a substratum to discern between different molecules on the cell surface [21]
Mechanosensing	Flagellar appendages of bacteria act as mechanosensors by sensing their rotation inhibition upon surface attachment. Pilus retraction also plays a mechanosensing role [22]

[Table/Fig-2]: Classification of microbial adhesion [20-22].

Methods to test microbial adhesion: The gold standard for quantitative bacterial detection is plate counting, often known as Colony Forming Unit (CFU) counts. However, it takes several days to cultivate bacteria, colony aggregation causes errors in detection and it is impossible to immediately detect bacterial attachment sites; and materials with irregular shapes or varied textures cannot be evaluated as a whole [23]. Alternative methods include Adenosine Triphosphate (ATP) bioluminescence, a non destructive bioluminescence technology, and crystal violet staining. While non destructive bioluminescence technology uses bacteria engineered with the luciferase gene to monitor bioluminescence signals in real-time, ATP bioluminescence assays rely on the oxidation reaction

between ATP and fluorescein in living cells to emit fluorescence, indirectly determining the number of adherent bacteria through the proportion between luminous intensity and microbial count [24]. Methods to detect microbial adhesion are shown in [Table/Fig-3] [21,25-30].

Quantitative methods		Qualitative methods	
Colony forming units	Method for quantifying bacteria: The pour-plate and surface-spread methods [25].	Crystal violet staining	It is a type of spectrophotometry; It is based on the quantitative relation between optical density and colony counts which is obtained from standard curves prepared for each bacterial species [25] It is to evaluate the early stages of bacterial biofilm formation [26].
16S rRNA gene sequencing	Accurate identification of microorganisms [27].	Fluorescent microscopy	Immunofluorescent method and observed under a microscope and are visualised using dyes.
Polymerase Chain Reaction (PCR)	Used for quantifying adhesion to intestinal epithelial cells.	Confocal scanning microscopy	Allows real-time visualisation of fully hydrated, living specimens [28] enable quantification of the forces involved in the adhesion of microbes on a single cell basis [21,29,30].
ATP bioluminescence	To assess bacterial adhesion with hydrophobic polystyrene tubes as the attachment surface.	Scanning Electron Microscopy (SEM)	For morphological assessment of bacteria adhered on a surface, the morphology of the material surface, and the relationships between them.

[Table/Fig-3]: Methods to detect microbial adhesion [21,25-30].

Changes in Oral Microbiome with Fixed Orthodontic Appliances

When a patient undergoes orthodontic treatment with fixed appliances, there is alteration in the microbiota, microbes initially adhere to the brackets and tissue surfaces of removable appliances, leading to biofilm formation [31]. There are numerous studies on interaction of oral bacteria with orthodontic brackets, but specific bracket type that favours biofilm development is unknown [3,32,33]. Ceramic brackets have been implicated to have more plaque retention than metal brackets but it remains controversial [33,34]. In a study by Abutayem H et al., seven different bracket systems were assessed for microbial adhesion. *E. coli* had the highest concentration in ceramic brackets [35]. Highest concentration of *S. mutans* was observed in self-ligating brackets, while *E. faecalis* was most common in SS wires [35].

Microbial Adhesion on Different Aligner Materials

In recent years, there has been an increased demand for aesthetic, metal-free orthodontic devices called aligners due to their less visibility and aesthetic appeal. These are recommended to be worn for 20-22 hours daily. Invisalign aligner, made from a polyurethane-based material, was introduced in the late 1990s is one of the most popular aligner systems used worldwide [36]. A systematic review by Ajwa N et al., reported that the overall changes of oral microbiota are higher in patients with fixed appliances than removable appliances [37]. Even though clear aligner therapy is associated with better oral hygiene levels in the short term there are concerns of plaque formation and WSL occurrence [38]. The included studies on microbial adhesion on clear aligners and cleaning methods of clear aligners [Table/Fig-4] [39-45].

Tektas S et al., reported no significant differences in the biofilm formation [39]. Rouzi M et al., and Sfondirini MF et al., observed microbial shifts, with a notable increase in *Streptococcus* spp [41-43]. While Yazdi M et al., observed that *C.albicans* formed the most

Authors	Intervention	Method of assessment	Organisms tested	Outcomes assessed	Outcome
Tektas S et al., 2020 [39]	CA-medium, copolyester, Duran, Erkodur Comparison - enamel surfaces and metal brackets	Colony forming units of aerobic and anaerobic bacteria	Both aerobic and anaerobic organisms	Bacterial adhesion at 2 hours and biofilm formation after 72 hours	No differences in initial microbial attachment and biofilm formation of aerobic and anaerobic species.
Bozkurt A et al., 2024 [40]	Invisalign, Clarity, Clear Correct, Smartee, Orthero and Graphy	Microwell titre assay Crystal violet assay	<i>Streptococcus mutans</i> (ATCC 25175) and <i>Lactobacillus acidophilus</i> (ATCC 4356)	Time-dependent biofilm formation and microbial adhesion	More biofilm formed on Clear Correct at 168 hours. <i>Streptococcus mutans</i> formed more biofilm on Invisalign at 240 hours <i>Lactobacillus acidophilus</i> bacteria formed significantly more biofilm on Invisalign at 240 hours.
Rouzi M et al., 2022 [41]	15 patients treated with invisalign aligners Aligner tray plaque and subgingival plaque was tested	16S rRNA Gene Amplicon Sequencing Sequence analysis	Plaque collected and tested	Plaque Index (PI), salivary flow, PD, BI, sequence analysis, microbial composition and diversity	Abundance of <i>Streptococcus</i> increased, as well as the richness and microbiota decreased substantially as the duration of treatment time.
Baybekov O et al., 2023 [42]	A microbiological examination of 10 persons using aligners obtained from adolescents aged 13 to 19 years PETG Aligner	1. CFUs 2. Crystal violet staining - OD values for biofilm formation 3. Confocal laser scanning microscopy	Used aligners were subjected to microbial isolation and testing		13 Gram-positive and 13 Gram-negative bacteria, and 2 yeast-like fungi. There are currently no means of ensuring the direct and complete destruction of biofilms.
Sfondrini MF et al., 2021 [43]	Intervention- clear aligner Control - no treatment	Real Time PCR-based test	Aggregatibacter actinomycetemcomitans, Tannerella forsythensis, Porphyromonas gingivalis, Troponema denticola, Prevotella intermedia and Fusobacterium nucleatum	Probing Pocket Depth (PPD), Bleeding on Probing (BOP) and Plaque Index (PI), and microbiological tests	Porphyromonas gingivalis and Prevotella intermedia increased in both groups. Conversely, Tannerella forsythia and Fusobacterium nucleatum increased in the clear aligner group. During the first two months of treatment, CAT has no discernible effect on periodontal and microbiological markers as compared to patients who are not receiving treatment.
Yazdi M et al., 2024 [14]	3D printed CAD/CAM material (Detax) and four thermoformed retainers Erkodent; EasyVac; DB and Clear Tech	Microtiter plate assay	<i>Streptococcus mutans</i> <i>Streptococcus sanguinis</i> , <i>Staphylococcus epidermidis</i> , <i>Staphylococcus aureus</i> , and <i>Lactobacillus casei</i>	Biofilm formation on 5 thermoformed and 3D printed CAD/CAM orthodontic retainers in 3 intervals	<i>Candida albicans</i> biofilm was more intense.
Mummolo S et al., 2019 [44]	30 subjects (aged 21.5±1.5 years) were treated with removable clear aligners (CA), while for other 30 cases (aged 23.3±1.6 years) a fixed multibrackets appliance (MB)	CRT® bacteria CFU	<i>S. mutans</i> and <i>Lactobacilli</i>	Investigate salivary concentrations of <i>S. mutans</i> and some Lactobacilli, and Plaque Index (PI) in patients wearing fixed versus removable orthodontic appliances	Only about 10% of CA patients and 13.3% of RP patients achieved a microbial colonisation which may lead to high risk of caries development, about 40% of MB patients - and 20% after 3 months - showed a high level of vulnerability to developing caries.
Levrini L et al., 2015 [45]	Invisalign® group, fixed orthodontic appliances group and control group	Total biofilm mass and periodontal pathogens were analysed and detected via real-time PCR	<i>P. intermedia</i> , <i>A. actinomycetemcomitans</i> , <i>P. gingivalis</i> , <i>Tannerella forsythia</i>	Total biofilm mass and periodontopathic bacterial species Periodontal indices	The microbiological analyses detected the presence of <i>A. actinomycetemcomitans</i> only in one patient treated with fixed orthodontic appliances.

[Table/Fig-4]: Characteristics of included studies [39-45,65].

biofilm formation with Detax retaining the least biofilm, followed by Erkodent and Clear Tech [14]. Baybekov O et al., observed that the biofilm formation varied based on the type of aligner, with clear correct aligner exhibiting higher count of *S. mutans* while Invisalign showed more biofilm than Clarity and Orthero. Additionally, Graphy demonstrated increased biofilm formation when *S. mutans* and *L. acidophilus* were combined. In a study by Baybekov O et al., results revealed that 28 different microbial species were isolated from the aligners, with a predominance of *Bifidobacterium* spp., *Candida albicans*, *E. coli*, and various *Streptococcus* and *Peptostreptococcus* species [42]. These species were found in significant concentrations (≥ 5 lg CFU/1 mL), indicating their dominance in the oral microflora. A systematic review by España-Pamplona P et al., analysed the impact of orthodontic appliances, including clear aligners, on oral microbiota and periodontal health. It found that clear aligners tend to promote a healthier oral microbiota, with improved periodontal indicators such as lower plaque and bleeding indices compared to fixed appliances [46].

In a review by Rouzi M et al., it was observed that clear aligners show reduced incidence of WSLs compared to fixed appliances. This could be attributed to their simplified oral hygiene routines, leading to lower plaque accumulation [41]. No significant changes

observed in bleeding on probing or probing depth in aligner patients. Improper cleaning of aligners can lead to the growth of harmful bacteria, underlining the importance of regular cleaning, the authors recommend a multistep cleaning approach that combines mechanical and chemical methods for optimal hygiene [47].

Properties of Aligners Influencing Microbial Adhesion

Microbial adhesion to clear aligners is influenced by surface properties, material composition, and intraoral conditions. Over time, aligner surfaces may undergo changes such as increased roughness and hydrolytic degradation, potentially enhancing bacterial colonisation. Surface properties such as roughness and wettability are linked to microbial adhesion [48,49]. Understanding these factors is crucial for maintaining aligner efficacy and minimising microbial accumulation through proper hygiene and regular dental check-ups. Surface properties such as roughness and wettability are linked to microbial adhesion [48,49]. Methods to reduce microbial adhesion on clear aligners [Table/Fig-5].

The changes associated with the intraoral aging of aligners, have been evaluated with respect to mechanical properties and surface roughness [50]. Hydrolytic degradation has been described as the main mechanism of the disintegration of polymers exposed

Mechanical methods	Chemical methods
• Brushing with toothpaste • Brushing with toothpaste and chlorhexidine • Sonic or ultrasonic bath	• Aligner cleaning crystals (e.g., Invisalign Cleaning crystals, retainer brite) • Tablets • Brushing with toothpaste+effervescent tablet • Rinsing with water • Cupral ®, a copper-calcium hydroxide solution

[Table/Fig-5]: Methods to reduce microbial adhesion on clear aligners.

to aqueous media [51]. Ihssen BA et al., demonstrated that water absorption can reduce the material's stiffness and may therefore promote a decrease in the orthodontic forces in PETG aligners [52].

Consequences of Microbial Adhesion - WSL Incidence

WSLs are clinically defined as opaque, white areas caused by the subsurface loss of enamel minerals [53]. WSL incidence was observed to be less in patients treated with CAT than traditional braces, which partially due to shorter treatment duration, or better pretreatment oral hygiene [54]. Approximately, 1.2% of the aligner patients developed WSLs, compared to 26% of the traditionally treated patients [55,56]. In a study by Liu Q et al., 35.5% of patients developed WSL during CAT. The incidence was higher in maxillary dentition with the lesions appearing symmetrical side to side. The order of incidence of WSL is as follows- maxillary lateral incisor, canine, central incisors, first premolars, second premolars and the first molars [57].

The variation in WSL incidence among studies could be attributed to differences in number of teeth examined, the methods and standardisation of the examinations, the location of the study sample, initial age, treatment duration, type orthodontic therapy, supplemental fluoride application and orthodontic materials [56]. In a meta-analysis by Llera-Romero, it was reported that, at the end of treatment (9-24 months), aligners patients have ten times less risk of presenting new WSLs than conventional fixed appliance patients [58].

In a study by Song Z et al., it was observed that the relative abundances of 14 taxa were significantly higher in the WSL group [59]. After clear aligner therapy, there were changes observed in the oral microenvironment, including inflammatory cytokine levels such as CXCLs and ILs, with CXCL8 expression significantly associated with the occurrence of WSLs [60].

Consequence Of Microbial Adhesion - Periodontal Problems

Periodontal health is defined by the absence of detectable signs of inflammation that may affect periodontal physiology [61]. Orthodontic appliances can promote plaque retention and a change in the composition of the oral microbiota, with possible effects on periodontal health and risk of caries. Some authors reported a different microbiota composition in plaque or saliva in aligner patients compared to patients wearing FAs [62,63]. This may be attributed to the fact that aligners are removable, allowing the maintenance of good oral hygiene.

During the assessment of periodontal parameters, aligners demonstrated significantly lower scores of plaque indices [64]. In a meta-analysis, it was observed that clear aligners were better for periodontal health than fixed appliances and might be recommended for patients at high-risk of developing gingivitis [45,65]. In another systematic review by Abay F et al., it was reported that the percentage of patients who developed new WSLs after clear aligner therapy varied between 1.2% and 41.18%, with a heterogeneity in the included articles.

Methods to Reduce Microbial Adhesion on Clear Aligners

There is possibility for adhesion and growth of bacterial biofilm in clear aligners though they are removable type of orthodontic

appliances. There are several methods to clean clear aligners. In a study by Levri L et al., it was observed that brushing with toothpaste alone is more efficient than rinsing with water, while better results are obtained when brushing with toothpaste is combined with an effervescent tablet [66]. Ultrasonic bath was observed to be efficient in removing bacterial biofilms from aligners. However, the SEM pictures obtained showed that the surface of the aligner was damaged by the ultrasonic vibrations, showing signs of ultrasonic cavitation [67].

In a trial by Shpack N et al., it was observed that the use of vibrating bath along with Cleaning Crystal solution was observed to be more efficient than using toothpaste and chlorhexidine to brush, but it did not exhibit bacterial inhibition zone [68]. The cleaning crystals used in the vibrating bath is composed of Sodium Sulfate, Sodium Carbonate, Sodium Tripolyphosphate, Sodium Dichloroisocyanurate and Sodium lauryl sulfate, which act as a pH neutraliser (e.g., Sodium Sulfate), disinfectant (e.g., Sodium Dichloroisocyanurate) and detergent (e.g., Sodium lauryl sulfate).

In a study by Levri L et al., patients were instructed to follow different aligner cleaning procedures for six weeks rinsing with water, soaking with cleaning tablets and brushing, and brushing with a soft toothbrush and toothpaste [66]. Scanning Electron Microscopy (SEM) analysis of the external and internal surfaces of aligners revealed that those cleaned using a combination of cleaning tablets and brushing were significantly cleaner compared to those rinsed with only water. Despite this, bacterial contamination, predominantly composed of spheroidal microorganisms, was observed on all aligners, with occasional mineral crystal deposits. The internal surfaces exhibited higher contamination levels, likely due to their concave shape, which made thorough cleaning more difficult.

Meto A et al., investigated the disinfectant properties of Cupral®, on aligners contaminated with biofilm and found it effective in eliminating bacteria [69]. Additionally, studies by Zhang M et al., and Xie Y et al., demonstrated that applying a gold nanoparticle coating to the aligner surface reduced initial bacterial adhesion, thereby preventing biofilm formation [70,71]. In-vitro findings were particularly promising, showing significant suppression of *Porphyromonas gingivalis*, a periodontopathogenic bacterium, and *Streptococcus mutans*, a key contributor to dental caries.

CONCLUSION(S)

Streptococcus mutans and *Lactobacillus* species have been observed to be the most common organisms linked to biofilm formation. The incidence of WSL has been linked to biofilm formation, leading to enamel demineralisation. Use of Cleaning Crystals for cleaning aligners has been reported to be more effective in eliminating bacterial contamination.

REFERENCES

- [1] Gibbons RJ. Bacterial adhesion to oral tissues: A model for infectious diseases. *J Dent Res* [Internet]. 1989;68(5):750-60. [cited 2025 Jan 19]. Available from: <https://journals.sagepub.com/doi/10.1177/00220345890680050101>.
- [2] 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* [Internet]. 2011;33(5):577-83. Available from: <http://dx.doi.org/10.1093/ejo/cjq126>.
- [3] Mulimani P, Popowics T. Effect of orthodontic appliances on the oral environment and microbiome. *Front Dent Med*. 2022;3:924835. Doi: 10.3389/fdmed.2022.924835.
- [4] Contaldo M, Lucchese A, Lajolo C, Rupe C, Di Stasio D, Romano A, et al. The oral microbiota changes in orthodontic patients and effects on oral health: An overview. *J Clin Med* [Internet]. 2021;10(4):780. [cited 2025 Jan 7]. Available from: <https://www.mdpi.com/2077-0383/10/4/780>.
- [5] Ogaard B, Rolla G, Arends J. Orthodontic appliances and enamel demineralization. Part 1. Lesion development. *Am J Orthod Dentofacial Orthop* [Internet]. 1988;94(1):68-73. [cited 2024 Dec 17]. Available from: [http://dx.doi.org/10.1016/0889-5406\(88\)90453-2](http://dx.doi.org/10.1016/0889-5406(88)90453-2).
- [6] Butera A, Maiorani C, Morandini A, Simonini M, Morittu S, Trombini J, et al. Evaluation of children caries risk factors: A narrative review of nutritional aspects, oral hygiene habits, and bacterial alterations. *Children* [Internet]. 2022;9(2):262. [cited 2024 Dec 17]. Available from: <https://www.mdpi.com/2227-9067/9/2/262>.

- [7] Lundström F, Krasse B. Streptococcus mutans and lactobacilli frequency in orthodontic patients; the effect of chlorhexidine treatments. *Eur J Orthod* [Internet]. 1987;9(2):109-16. Available from: <http://dx.doi.org/10.1093/ejo/9.2.109>.
- [8] Eliades T, Eliades G, Brantley WA. Microbial attachment on orthodontic appliances: I. Wettability and early pellicle formation on bracket materials. *Am J Orthod Dentofacial Orthop* [Internet]. 1995;108(4):351-60. Available from: [http://dx.doi.org/10.1016/s0889-5406\(95\)70032-3](http://dx.doi.org/10.1016/s0889-5406(95)70032-3).
- [9] 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* [Internet]. 2021;78(2):566-75. [cited 2025 Apr 26]. Available from: <https://link.springer.com/article/10.1007/s00284-020-02308-5>.
- [10] Kim SH, Choi DS, Jang I, Cha BK, Jost-Brinkmann PG, Song JS. Microbiologic changes in subgingival plaque before and during the early period of orthodontic treatment. *Angle Orthod* [Internet]. 2012;82(2):254-60. Available from: <http://dx.doi.org/10.2319/030311-156.1>.
- [11] Pan S, Liu Y, Si Y, Zhang Q, Wang L, Liu J, et al. Prevalence of fimA genotypes of Porphyromonas gingivalis in adolescent orthodontic patients. *PLoS One* [Internet]. 2017;12(11):e0188420. [cited 2025 Jan 7]. Available from: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0188420&type=printable>.
- [12] Rosvall MD, Fields HW, Ziuchkovski J, Rosenstiel SF, Johnston WM. Attractiveness, acceptability, and value of orthodontic appliances. *Am J Orthod Dentofacial Orthop* [Internet]. 2009;135(3):276.e1-12; discussion 276-77. Available from: <http://dx.doi.org/10.1016/j.ajodo.2008.09.020>.
- [13] Chen W, Chen J, Bai D, Wang P, Shu R. Effects of clear aligners and traditional removable appliances on oral microbiome in mixed dentition: A comparative study. *BMC Oral Health* [Internet]. 2024;24(1):1276. Available from: <http://dx.doi.org/10.1186/s12903-024-05063-2>.
- [14] Yazdi M, Daryanavard H, Ashtiani AH, Moradinejad M, Rakhshan V. A systematic review of biocompatibility and safety of orthodontic clear aligners and transparent vacuum-formed thermoplastic retainers: Bisphenol-A release, adverse effects, cytotoxicity, and estrogenic effects. *Dent Res J (Isfahan)* [Internet]. 2023;20:41. [cited 2025 Jan 5]. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC10166753/>.
- [15] Alghamdi S. Isolation and identification of the oral bacteria and their characterization for bacteriocin production in the oral cavity. *Saudi J Biol Sci* [Internet]. 2022;29(1):318-23. [cited 2025 Feb 2]. Available from: <http://dx.doi.org/10.1016/j.sjbs.2021.08.096>.
- [16] Souza PRM, Dupont L, Mosena G, Dantas ML, Bulcão LA. Variations of oral anatomy and common oral lesions. *Anais Brasileiros de Dermatologia* [Internet]. 2024;99(1):03-18. [cited 2025 Jan 19]. Available from: <http://dx.doi.org/10.1016/j.abd.2023.06.001>.
- [17] Tezal M, Scannapieco FA, Wactawski-Wende J, Grossi SG, Genco RJ. Supragingival plaque may modify the effects of subgingival bacteria on attachment loss. *J Periodontol* [Internet]. 2006;77(5):808-13. [cited 2025 Jan 30]. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1902/jop.2006.050332>.
- [18] Zheng S, Bawazir M, Dhall A, Kim HE, He L, Heo J, et al. Implication of surface properties, bacterial motility, and hydrodynamic conditions on bacterial surface sensing and their initial adhesion. *Front Bioeng Biotechnol* [Internet]. 2021;9:643722. Available from: <http://dx.doi.org/10.3389/fbioe.2021.643722>.
- [19] Alam F, Kumar S, Varadarajan KM. Quantification of adhesion force of bacteria on the surface of biomaterials: Techniques and assays. *ACS Biomater Sci Eng* [Internet]. 2019;5(5):2093-110. Available from: <http://dx.doi.org/10.1021/acsbomaterials.9b00213>.
- [20] Straub H, Bigger CM, Valentin J, Abt D, Qin XH, Eberl L, et al. Bacterial adhesion on soft materials: Passive physicochemical interactions or active bacterial mechanosensing? *Adv Healthc Mater* [Internet]. 2019;8(8):1801323. [cited 2024 Dec 17]. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1002/adhm.201801323>.
- [21] Busscher HJ, Weerkamp AH. Specific and non-specific interactions in bacterial adhesion to solid substrata. *FEMS Microbiol Lett* [Internet]. 1987;46(2):165-73. Available from: <https://academic.oup.com/femsre/article-lookup/doi/10.1111/j.1574-6968.1987.tb02457.x>.
- [22] Persat A. Bacterial mechanotransduction. *Curr Opin Microbiol* [Internet]. 2017;36:01-06. [cited 2024 Dec 17]. Available from: <http://dx.doi.org/10.1016/j.mib.2016.12.002>.
- [23] Wang L, Qiao X, Gao L, Chen C, Wan Y. A quantitative method to assess bacterial adhesion using recombinant bioluminescent Pseudomonas aeruginosa. *swmxb* [Internet]. 2021;7(1):55-70. [cited 2025 Jan 19]. Available from: <http://www.biophysics-reports.org/en/article/doi/10.52601/bpr.2021.200043?viewType=HTML>.
- [24] Braissant O, Astasov-Frauenhoffer M, Waltimo T, Bonkat G. A review of methods to determine viability, vitality, and metabolic rates in microbiology. *Front Microbiol* [Internet]. 2020;11:547458. [cited 2025 Jan 20]. Available from: <https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2020.547458/pdf>.
- [25] An YH, Friedman RJ. Laboratory methods for studies of bacterial adhesion. *Journal of Microbiological Methods* [Internet]. 1997;30(2):141-52. [cited 2025 Feb 2]. Available from: [http://dx.doi.org/10.1016/S0167-7012\(97\)00058-4](http://dx.doi.org/10.1016/S0167-7012(97)00058-4).
- [26] Borowicz M, Krzyżanowska DM, Jafra S. Crystal violet-based assay for the assessment of bacterial biofilm formation in medical tubing. *J Microbiol Methods* [Internet]. 2023;204:106656. Available from: <http://dx.doi.org/10.1016/j.mimet.2022.106656>.
- [27] Petti CA, Polage CR, Schreckenberger P. The role of 16S rRNA Gene sequencing in identification of microorganisms misidentified by conventional methods. *J Clin Microbiol* [Internet]. 2005;43(12):6123-25. [cited 2025 Feb 2]. Available from: <https://journals.asm.org/doi/10.1128/jcm.43.12.6123-6125.2005>.
- [28] Schlafer S, Meyer RL. Confocal microscopy imaging of the biofilm matrix. *J Microbiol Methods* [Internet]. 2017;138:50-59. Available from: <http://dx.doi.org/10.1016/j.mimet.2016.03.002>.
- [29] Dufrêne YF. Sticky microbes: Forces in microbial cell adhesion. *Trends Microbiol* [Internet]. 2015;23(6):376-82. Available from: <http://dx.doi.org/10.1016/j.tim.2015.01.011>.
- [30] Manning PA. Use of confocal microscopy in studying bacterial adhesion and invasion. *Methods Enzymol* [Internet]. 1995;253:159-66. Available from: [http://dx.doi.org/10.1016/s0076-6879\(95\)53016-9](http://dx.doi.org/10.1016/s0076-6879(95)53016-9).
- [31] Perkowski K, Baltaza W, Conn DB, Marczyńska-Stolarek M, Chomicz L. Examination of oral biofilm microbiota in patients using fixed orthodontic appliances in order to prevent risk factors for health complications. *Ann Agric Environ Med* [Internet]. 2019;26(2):231-35. Available from: <http://dx.doi.org/10.26444/aaem/105797>.
- [32] Fournier A, Payant L, Bouclin R. Adherence of Streptococcus mutans to orthodontic brackets. *Am J Orthod Dentofacial Orthop* [Internet]. 1998;114(4):414-17. Available from: [http://dx.doi.org/10.1016/s0889-5406\(98\)70186-6](http://dx.doi.org/10.1016/s0889-5406(98)70186-6).
- [33] Ahn SJ, Kho HS, Lee SW, Nahm DS. Roles of salivary proteins in the adherence of oral streptococci to various orthodontic brackets. *J Dent Res* [Internet]. 2002;81(6):411-15. Available from: <http://dx.doi.org/10.1177/154405910208100611>.
- [34] Lindel ID, Elter C, Heuer W, Heidenblut T, Stiesch M, Schweska-Polly R, et al. Comparative analysis of long-term biofilm formation on metal and ceramic brackets. *Angle Orthod* [Internet]. 2011;81(5):907-14. Available from: <http://dx.doi.org/10.2319/102210-616.1>.
- [35] Abutayem H, Abdullatif Alshehri M, Alameri M, Sohail Zafar M. Microbial adhesion on different types of orthodontic brackets and wires: An in-vitro study. *Saudi Dent J* [Internet]. 2024;36(11):1459-65. Available from: <http://dx.doi.org/10.1016/j.sdentj.2024.09.004>.
- [36] Weir T. Clear aligners in orthodontic treatment. *Aust Dent J* [Internet]. 2017;62:58-62. [cited 2025 Apr 26]. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/adj.12480>.
- [37] Ajwa N, AlRashoud R, AlWehaibi J, AlMazyad A. Orthodontic clear aligner vs fixed appliances' influence on oral Microbiota and salivary parameters': A systematic review. *Ann Dent Spec* [Internet]. 2021;9(4):16-22. Available from: <https://annalsofdentalspecialty.net.in/article/orthodontic-clear-aligner-vs-fixed-appliances-influence-on-oral-microbiota-and-salivary-parameters-tdklojhm3qinw>.
- [38] Oikonomou E, Foros P, Tagkli A, Rahiotis C, Eliades T, Koletsis D. Impact of aligners and fixed appliances on oral health during orthodontic treatment: A systematic review and meta-analysis. *Oral Health Prev Dent* [Internet]. 2021;19(1):659-72. [cited 2024 Nov 18]. Available from: https://www.zora.uzh.ch/id/eprint/216294/8/ohpd_2021_01_s0659.pdf.
- [39] Tektas S, Thurnheer T, Eliades T, Attin T, Karygianni L. Initial bacterial adhesion and biofilm formation on aligner materials. *Antibiotics (Basel)* [Internet]. 2020;9(12):908. Available from: <http://dx.doi.org/10.3390/antibiotics9120908>.
- [40] Pasaoglu Bozkurt A, Demirci M, Erdogan P, Kayalar E. Comparison of microbial adhesion and biofilm formation on different orthodontic aligners. *Am J Orthod Dentofacial Orthop* [Internet]. 2025;167(1):47-62. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0889540624003615>.
- [41] Rouzi M, Jiang Q, Zhang H, Li X, Long H, Lai W. Characteristics of oral microbiota and oral health in the patients treated with clear aligners: A prospective study. *Clin Oral Investig* [Internet]. 2023;27(11):6725-34. Available from: <http://dx.doi.org/10.1007/s00784-023-05281-y>.
- [42] Baybekov O, Stanishevskiy Y, Sachivkina N, Bobunova A, Zhabo N, Avdonina M. Isolation of clinical microbial isolates during orthodontic aligner therapy and their ability to form biofilm. *Dent J (Basel)* [Internet]. 2023;11(1):13. Available from: <http://dx.doi.org/10.3390/dj11010013>.
- [43] Sfondrini MF, Butera A, Di Michele P, Luccisano C, Ottini B, Sangalli E, et al. Microbiological changes during orthodontic aligner therapy: A prospective clinical trial. *Appl Sci* [Internet]. 2021;11(15):6758. [cited 2025 Jan 20]. Available from: <https://www.mdpi.com/2076-3417/11/15/6758>.
- [44] Mummolo S, Tieri M, Nota A, Caruso S, Darvizeh A, Albani F, 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* [Internet]. 2020;6(2):181-87. [cited 2025 Jan 20]. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1002/cre2.261>.
- [45] 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* [Internet]. 2016;2016(1):5926941. [cited 2025 Jan 5]. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1155/2016/5926941>.
- [46] España-Pamplona P, Bernés-Martínez L, Andrés-Castelló C, Bolás-Colveé B, Adobes-Martín M, Garcovich D. Changes in the oral microbiota with the use of aligners vs. braces: A systematic review. *J Clin Med* [Internet]. 2024;13(23):7435. [cited 2025 Jan 20]. Available from: <https://www.mdpi.com/2077-0383/13/23/7435>.
- [47] Rouzi M, Zhang X, Jiang Q, Long H, Lai W, Li X. Impact of clear aligners on oral health and oral microbiome during orthodontic treatment. *Int Dent J* [Internet]. 2023;73(5):603-11. [cited 2025 Jan 20]. Available from: <http://dx.doi.org/10.1016/j.identj.2023.03.012>.
- [48] Alexandropoulos A, Al Jabbari YS, Zinelis S, Eliades T. Chemical and mechanical characteristics of contemporary thermoplastic orthodontic materials. *Aust Orthod J* [Internet]. 2015;31(2):165-70. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/26999889>.
- [49] Suter F, Zinelis S, Patcas R, Schätzle M, Eliades G, Eliades T. Roughness and wettability of aligner materials. *J Orthod* [Internet]. 2020;47(3):223-31. [cited 2025 Jan 5]. Available from: <https://journals.sagepub.com/doi/10.1177/1465312520936702>.

- [50] Papadopoulou AK, Cantele A, Polychronis G, Zinelis S, Eliades T. Changes in roughness and mechanical properties of invisalign® appliances after one- and two-weeks use. *Materials* [Internet]. 2019;12(15):2406. [cited 2025 Jan 5]. Available from: <https://www.mdpi.com/1996-1944/12/15/2406>.
- [51] Mondal S, Martin D. Hydrolytic degradation of segmented polyurethane copolymers for biomedical applications. *Polymer Degradation and Stability* [Internet]. 2012;97(8):1553-61. [cited 2025 Jan 5]. Available from: <http://dx.doi.org/10.1016/j.polydegstab.2012.04.008>.
- [52] Ihssen BA, Willmann JH, Nimer A, Drescher D. Effect of in vitro aging by water immersion and thermocycling on the mechanical properties of PETG aligner material. *J Orofac Orthop* [Internet]. 2019;80(6):292-303. Available from: <http://dx.doi.org/10.1007/s00056-019-00192-8>.
- [53] Huang GJ, Roloff-Chiang B, Mills BE, Shalchi S, Spiekerman C, Korpak AM, et al. Effectiveness of MI paste plus and previDent fluoride varnish for treatment of white spot lesions: A randomized controlled trial. *Am J Orthod Dentofacial Orthop* [Internet]. 2013;143(1):31-41. Available from: <http://dx.doi.org/10.1016/j.ajodo.2012.09.007>.
- [54] Abay F, Kutalmış Buyuk S, Korkmaz YN. Prevalence of white spot lesions during clear aligner therapy: A systematic review. *Australasian Orthodontic Journal* [Internet]. 2022;38(2):368-79. Available from: <https://www.sciendo.com/article/10.2478/aoj-2022-0035>.
- [55] 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* [Internet]. 2019;89(3):359-64. Available from: <http://dx.doi.org/10.2319/073118-553.1>.
- [56] Azeem M, Ul Hamid W. Incidence of white spot lesions during orthodontic clear aligner therapy. *J World Fed Orthod* [Internet]. 2017;6(3):127-30. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S2212443817300735>.
- [57] Liu Q, Song Z. Incidence, severity, and risk factors for white spot lesions in adolescent patients treated with clear aligners. *Orthod Craniofac Res* [Internet]. 2024;27(5):704-13. [cited 2025 Feb 3]. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/ocr.12791>.
- [58] Llera-Romero AS, Adobes-Martín M, Iranzo-Cortés JE, José María Montiel-Company, Garcovich D. Periodontal health status, oral microbiome, white-spot lesions and oral health related to quality of life-clear aligners versus fixed appliances: A systematic review, meta-analysis and meta-regression. *Korean J Orthod* [Internet]. 2023;53(6):374-92. [cited 2025 Feb 3]. Available from: <https://e-kjo.org/journal/view.html?doi=10.4041/kjod22.272>.
- [59] Song Z, Fang S, Guo T, Wen Y, Liu Q, Jin Z. Microbiome and metabolome associated with white spot lesions in patients treated with clear aligners. *Front Cell Infect Microbiol* [Internet]. 2023;13:1119616. Available from: <http://dx.doi.org/10.3389/fcimb.2023.1119616>.
- [60] Liu Q, Guo T, Dang W, Song Z, Wen Y, Luo H, et al. Correlation between salivary cytokine profiles and white spot lesions in adolescent patients receiving clear aligner orthodontic treatment. *BMC Oral Health* [Internet]. 2023;23(1):857. Available from: <http://dx.doi.org/10.1186/s12903-023-03561-3>.
- [61] Lang NP, Bartold PM. Periodontal health. *J Periodontol* [Internet]. 2018;89:S9-S16. [cited 2025 Jan 5]. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1002/JPER.16-0517>.
- [62] Sifakakis I, Papaioannou W, Papadimitriou A, Kloukos D, Papageorgiou SN, Eliades T. Salivary levels of cariogenic bacterial species during orthodontic treatment with thermoplastic aligners or fixed appliances: A prospective cohort study. *Prog Orthod* [Internet]. 2018;19(1):25. Available from: <http://dx.doi.org/10.1186/s40510-018-0230-4>.
- [63] Karkhaneechi M, Chow D, Sipkin J, Sherman D, Boylan RJ, Norman RG, et al. Periodontal status of adult patients treated with fixed buccal appliances and removable aligners over one year of active orthodontic therapy. *Angle Orthod* [Internet]. 2013;83(1):146-51. Available from: <http://dx.doi.org/10.2319/031212-217.1>.
- [64] Miethke RR, Brauner K. A comparison of the periodontal health of patients during treatment with the invisalign system and with fixed lingual appliances. *J Orofac Orthop* [Internet]. 2007;68(3):223-31. [cited 2025 Jan 5]. Available from: <https://link.springer.com/article/10.1007/s00056-007-0655-8>.
- [65] Jiang Q, Li J, Mei L, Du J, Levrini L, Abbate GM, et al. Periodontal health during orthodontic treatment with clear aligners and fixed appliances: A meta-analysis. *J Am Dent Assoc* [Internet]. 2018;149(8):712-20.e12. Available from: <http://dx.doi.org/10.1016/j.adaj.2018.04.010>.
- [66] 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* [Internet]. 2015;7:125-31. Available from: <http://dx.doi.org/10.2147/CCIDE.S95814>.
- [67] Lombardo L, Martini M, Cervinara F, Spedicato GA, Oliverio T, Siciliani G. Comparative SEM analysis of nine F22 aligner cleaning strategies. *Prog Orthod* [Internet]. 2017;18(1):26. Available from: <http://dx.doi.org/10.1186/s40510-017-0178-9>.
- [68] Shpack N, Greenstein RBN, Gazit D, Sarig R, Vardimon AD. Efficacy of three hygienic protocols in reducing biofilm adherence to removable thermoplastic appliance. *Angle Orthod* [Internet]. 2014;84(1):161-70. Available from: <http://dx.doi.org/10.2319/012413-75.1>.
- [69] Meto A, Colombari B, Castagnoli A, Sarti M, Denti L, Blasi E. Efficacy of a copper-calcium-hydroxide solution in reducing microbial plaque on orthodontic clear aligners: A case report. *Eur J Dent* [Internet]. 2019;13(03):478-84. [cited 2025 Feb 6]. Available from: <http://www.thieme-connect.de/DOI/DOI?10.1055/s-0039-1695653>.
- [70] 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* [Internet]. 2020;6(2):1239-46. [cited 2025 Feb 6]. Available from: <https://pubs.acs.org/doi/abs/10.1021/acsbmaterials.9b01647>.
- [71] Zhang M, Liu X, Xie Y, Zhang Q, Zhang W, Jiang X, et al. Biological safe gold nanoparticle-modified dental aligner prevents the porphyromonas gingivalis biofilm formation. *ACS Omega* [Internet]. 2020;5(30):18685-92. [cited 2025 Feb 6]. Available from: <https://pubs.acs.org/doi/full/10.1021/acsomega.0c01532>.

PARTICULARS OF CONTRIBUTORS:

1. Postgraduate Student, Department of Orthodontics, SIMATS, Chennai, Tamil Nadu, India.
2. Professor, Department of Orthodontics, SIMATS, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Ravindra Kumar Jain,
Professor, Department of Orthodontics, Saveetha Dental College and Hospitals, 162,
Poonamallee High Road, Velappanchavadi, Chennai-600077, Tamil Nadu, India.
E-mail: ravindrakumar@saveetha.com

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Feb 08, 2025
- Manual Googling: Oct 23, 2025
- iThenticate Software: Oct 28, 2025 (9%)

ETYMOLOGY: Author Origin

EMENDATIONS: 8

Date of Submission: Feb 06, 2025

Date of Peer Review: Apr 04, 2025

Date of Acceptance: Nov 01, 2025

Date of Publishing: May 01, 2026