In Vitro Evaluation of Antimicrobial Mouthwashes on Biofilm Formed on Polyethylene Terephthalate Glycol–Based Orthodontic Template Aligner Materials

https://doi.org/10.70921/medev.v31i4.2016

Vlad Tiberiu Alexa[1,2], Stefania Dinu[3,4], Berivan Laura Rebeca Buzatu[1,2], Victor Emil Alexa[5], Ramona Dumitrescu[1,2], Ruxandra Sava-Rosianu[1,2], Atena Galuscan[1,2], Sorana Nicoleta Rosu[6]

[1]*Clinic of Preventive, Community Dentistry and Oral Health, Victor Babes University of Medicine and Phar-macy, Eftimie Murgu Sq. no 2, 300041 Timisoara, Romania*
[2]*Translational and Experimental Clinical Research Center in Oral Health (TEXC-OH), Department of Preventive, Community Dentistry and Oral Health, Victor Babes University of Medicine and Pharmacy 14A Tudor Vladimirescu Ave., 300173 Timisoara, Romania*
[3]*Department of Pedodontics, Faculty of Dental Medicine, "Victor Babeş" University of Medicine and Pharmacy, No. 9, Revolutiei 1989 Bv., 300041 Timisoara, Romania*
[4]*Pediatric Dentistry Research Center, Faculty of Dental Medicine, Victor Babeş University of Medicine and Pharmacy Timişoara, 9 No., Revolutiei Bv., 300041 Timişoara, Romania*
[5]*Victor Babeş University of Medicine and Pharmacy Timişoara, 9 No., Revolutiei Bv., 300041 Timişoara, Romania*
[6]*Department of Oral and Maxillofacial Surgery, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, 700115 Iasi, Romania*

Correspondence to:
Name: Stefania Dinu
E-mail address: dinu.stefania@umft.ro

Received: 27 November 2025; Accepted: 04 December 2025; Published: 15 December 2025

Abstract

Orthodontic template aligners used for attachment bonding are thermoformed polymeric materials that may act as vectors for microbial contamination despite their short intraoral use. This study evaluated the antimicrobial efficacy of seven mouthwash solutions on biofilms formed by Streptococcus mutans, Streptococcus oralis, and Candida albicans on a polyethylene terephthalate glycol–based template aligner material. After 24 h biofilm formation, samples were exposed to the tested mouthwashes for 1 min and microbial viability was assessed by optical density measurements. The solution containing fluoride and cetylpyridinium chloride showed the highest antibacterial activity, while the essential oil–based formulation exhibited the strongest antifungal effect. In contrast, fluoride-only solutions showed reduced efficacy, and one plant-based formulation demonstrated a slight stimulatory effect on Candida albicans. These findings indicate that the antimicrobial performance of mouthwashes on template aligner materials depends primarily on their chemical composition rather than fluoride content alone.

Keywords: Orthodontic template aligners; Oral biofilm; Streptococcus mutans; Streptococcus oralis; Candida albicans; Mouthwash; Antimicrobial activity.

INTRODUCTION

Clear aligner therapy has become an integral part of contemporary orthodontic practice due to its aesthetic advantages, improved comfort, and enhanced patient compliance compared with fixed appliances [1]. However, the prolonged intraoral wear of aligners, combined with frequent removal and reinsertion cycles, creates a favorable environment for microbial adhesion and biofilm maturation [2]. Inadequate hygiene during these cycles may facilitate the transfer of oral microorganisms onto the aligner surface, increasing the risk of enamel demineralization, gingival inflammation, and opportunistic fungal infections [3,4].

Among the wide spectrum of removable orthodontic devices, template aligners used for attachment placement represent a distinct category of thermoformed materials that come into direct contact with enamel and gingival surfaces during clinical procedures. Although these materials are typically used for short-term intraoral exposure, their intimate adaptation and repeated clinical handling expose them to oral microorganisms, making them a potential vector for bacterial and fungal contamination [5]. Despite this, template aligner materials have been relatively underrepresented in microbiological investigations, most studies focusing instead on treatment aligners intended for long-term wear. This highlights an important knowledge gap regarding microbial behavior and biofilm susceptibility on orthodontic template aligner surfaces [6].

Biofilm formation on polymer-based orthodontic materials is a dynamic, multistage process mediated by microbial adhesion, surface roughness, hydrophobicity, and surface free energy of the substrate [7]. Streptococcus mutans and Streptococcus oralis play key roles in the early colonization phases of oral biofilms, contributing to acidogenicity, enamel demineralization, and plaque maturation. In parallel, Candida albicans is a common opportunistic fungus frequently associated with appliance-related oral candidiasis, particularly in susceptible patients [7,8]. The synergistic interactions between bacterial and fungal species further enhance biofilm complexity, resistance, and pathogenic potential.

Polymer composition and surface characteristics significantly influence microbial adhesion and biofilm development. Thermoformed aligner materials based on copolyester polymers are characterized by relatively high surface energy and susceptibility to microstructural changes during fabrication, which can affect their interaction with oral microorganisms [9]. While polyurethane-based aligners have been shown to exhibit smoother topography and reduced biofilm affinity, copolyester-based materials may facilitate stronger microbial attachment due to greater surface heterogeneity. However, systematic data regarding biofilm formation and chemical decontamination on orthodontic template aligner materials remain scarce.

In clinical practice, chemical disinfection using mouthwashes represents a common and accessible approach for controlling microbial contamination of orthodontic appliances [10]. A wide range of formulations is available, including chlorhexidine, fluoride-containing rinses, essential-oil-based solutions, and plant-derived antiseptics [11]. Although these agents have demonstrated antimicrobial activity on dental hard tissues and conventional orthodontic appliances, their efficacy on orthodontic template aligner materials is not fully understood [12]. Moreover, the presence of polymer-specific interactions may influence the retention, activity, and diffusion of active compounds on these surfaces [13].

Aim and objectives

The present in vitro study aimed to evaluate the efficacy of several antiseptic and commercially available mouthwash solutions in reducing the viability of biofilms formed by Streptococcus mutans, Streptococcus oralis, and Candida albicans on standardized fragments

of a polyethylene terephthalate glycol–based orthodontic template aligner material. By simulating clinically relevant conditions of microbial colonization and short-term disinfectant exposure, this research seeks to provide objective, material-specific data that can support evidence-based recommendations for the chemical decontamination and safe handling of orthodontic template aligners in both clinical and laboratory settings.

MATERIAL AND METHODS

This in vitro study evaluated the antimicrobial efficacy of several commercially available mouthwash solutions on biofilms formed by Streptococcus mutans, Streptococcus oralis, and Candida albicans on a polyethylene terephthalate glycol–based orthodontic template aligner material.

Biofilms were allowed to develop over 24 h on standardized aligner fragments immersed in microbial suspensions [14]. After incubation, each sample was exposed for 1 min to one of seven tested mouthwash solutions. Residual microbial viability was assessed after a further 24 h of incubation, using spectrophotometric optical density measurements at 540 nm.

Microorganisms and Culture Conditions

Three representative oral microorganisms were used in this study: Streptococcus mutans, Streptococcus oralis, and Candida albicans. The strains were isolated from oral samples obtained from healthy volunteers and identified using routine microbiological procedures. Sample collection was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee (Aviz CECS Nr. 85/01.11.2021). Written informed consent was obtained from all participants prior to sampling.

Streptococcus mutans and Streptococcus oralis were cultured on Mitis Salivarius Agar supplemented with potassium tellurite and incubated anaerobically at 37 °C for 48 h. Candida albicans was cultured on Sabouraud Dextrose Agar and incubated aerobically at 37 °C for 24–48 h. Fresh colonies were used for the preparation of microbial suspensions.

Inoculum Preparation and Standardization

Microbial suspensions were prepared in sterile Brain Heart Infusion (BHI) broth. The turbidity of each suspension was adjusted to the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL.

Subsequently, standardized working dilutions were prepared as follows:

- 10^{-2} dilution for Streptococcus mutans and Streptococcus oralis
- 10^{-3} dilution for Candida albicans

These dilutions were used throughout the experimental protocol to ensure reproducibility and standardized microbial load.

Aligner Template Material and Sample Preparation

The investigated material consisted of a polyethylene terephthalate glycol–based orthodontic template aligner material, commonly used in clinical attachment bonding procedures. New, unused aligners were sectioned into standardized square fragments measuring 0.5 ± 0.05 cm, using a sterilizable metallic cutting mold to ensure dimensional uniformity. All fragments were decontaminated by immersion in 70% ethanol, rinsed with sterile distilled water, and dried under laminar airflow. Only unused materials were employed in order to eliminate any alteration of the surface morphology caused by intraoral aging or mechanical wear.

Biofilm Formation

Each aligner fragment was placed in an individual sterile test tube containing one of the microbial suspensions. The samples were incubated at 37 °C for 24 h to allow microbial adhesion and biofilm formation on the material surface. Fragments incubated in sterile BHI

without microorganisms served as negative controls. The presence of biofilm was confirmed on randomly selected samples using crystal violet staining.

Mouthwash Exposure Protocol

After the 24 h biofilm formation period, each fragment was retrieved under sterile conditions and immersed for 1 minute in 2 mL of the tested mouthwash solution. Seven commercially available mouthwashes were selected and anonymized as MW-A to MW-G, including formulations based on chlorhexidine, fluoride compounds, essential oils, and plant-derived agents. Following exposure, all samples were rinsed twice with sterile phosphate-buffered saline (PBS) to remove loosely attached microorganisms and residual liquid. No chemical neutralizing agent was used, in order to simulate realistic rinsing conditions encountered in clinical practice.

Post-Treatment Regrowth Assay

Each treated fragment was then transferred into sterile Eppendorf tubes containing 1 mL of fresh BHI broth and incubated for an additional 24 h at 37 °C. This step allowed the assessment of residual viable microorganisms based on their regrowth capacity after exposure to the tested solutions.

Spectrophotometric Analysis

After the 24 h regrowth period, microbial growth was quantified by measuring the optical density at 540 nm (OD_{540}) using a microplate spectrophotometer (Bio-Rad PR1100, Hercules, CA, USA). Sterile BHI broth was used as a blank, and untreated biofilm samples served as positive growth controls. All measurements were performed in triplicate, and the mean OD_{540} value was calculated for each group. Instrument calibration and baseline correction were performed prior to each measurement session to ensure reproducibility and accuracy.

Calculation of Growth and Inhibition Percentages

To determine antimicrobial efficacy, the following parameters were calculated:

1 Bacterial/Fungal Growth Percentage (BGP/FGP):

$$BGP = \frac{OD_{treatment}}{OD_{control}} \times 100$$

2 Bacterial/Fungal Inhibition Percentage (BIP/FIP):

$$BIP = 100 - BGP$$

where:

- $OD_{control}$ represents the optical density of untreated biofilm,
- $OD_{treatment}$ represents the optical density after mouthwash exposure.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Prior to statistical analysis, data normality was assessed using the Shapiro–Wilk test, and variance homogeneity was tested using Levene's test.

A two-way ANOVA was performed to evaluate the effect of mouthwash type on biofilm inhibition. When statistically significant differences were identified, Tukey's HSD post-hoc test with Copenhaver–Holland adjustment was applied for multiple comparisons. A significance level of $p < 0.05$ was adopted.

Statistical analysis was performed using PAST software (version 4.03).

RESULTS

The antimicrobial performance of the tested mouthwash solutions was evaluated based on the bacterial/fungal inhibition percentage (BIP/FIP%) against biofilms formed by Streptococcus mutans, Streptococcus oralis, and Candida albicans on polyethylene terephthalate glycol–based orthodontic template aligner material.

All data were processed using optical density measurements at 540 nm and expressed as inhibition percentages relative to untreated biofilm controls, as described in the Materials and Methods section.

Antibacterial Activity against *Streptococcus mutans*

Considerable differences in antibacterial efficacy were observed between the tested mouthwash solutions (Table 1).

The solution coded MW-B exhibited the highest inhibitory effect against S. mutans biofilm (BIP = 81.93%), followed by MW-G (76.14%) and MW-C (41.52%). In contrast, MW-E and MW-F demonstrated limited antibacterial activity, yielding inhibition values of 10.66% and 14.21%, respectively.

Notably, the high inhibition recorded for MW-B was associated with the presence of fluoride and cetylpyridinium chloride in its formulation, while MW-G, although fluoride-free, showed strong antibacterial activity, likely due to its essential oil content.

Full numerical data for all tested solutions are presented in Table 1.

Table 1. Microbial inhibition percentages (BIP/FIP%) of the tested mouthwash solutions

Mouthwash Code	*S. mutans* (%)	*S. oralis* (%)	*C. albicans* (%)
MW-A	24.47	10.86	14.99
MW-B	81.93	80.51	18.76
MW-C	41.52	21.83	2.31
MW-D	26.40	31.68	6.39
MW-E	10.66	−3.76	−13.21
MW-F	14.21	11.27	2.41
MW-G	76.14	29.44	33.54

Antibacterial Activity against *Streptococcus oralis*

For *Streptococcus oralis*, the most effective antibacterial solution was again MW-B (BIP = 80.51%). Moderate inhibition was observed for MW-D (31.68%) and MW-G (29.44%). In contrast, MW-E showed a negative inhibition value (−3.76%), indicating a potential stimulatory effect on biofilm development rather than suppression.

These results suggest a species-dependent response to the chemical formulations, with *S. oralis* showing higher resistance to several tested agents compared to *S. mutans*.

Detailed inhibition percentages are illustrated in Figure 1.

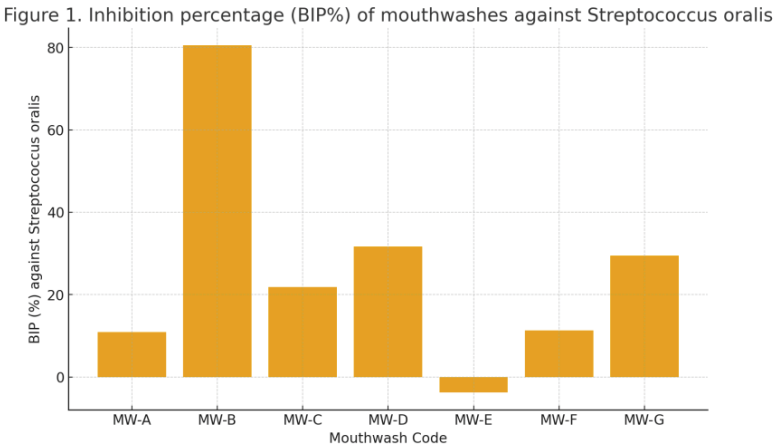

Figure 1. Inhibition percentages (BIP%) of the tested mouthwash solutions against Streptococcus oralis biofilm formed on polyethylene terephthalate glycol–based orthodontic template aligner material. Negative values indicate a potential stimulatory effect on biofilm development under the tested conditions.

Antifungal Activity against *Candida albicans*

The antifungal effects of the tested mouthwash solutions against *Candida albicans* biofilm formed on the polyethylene terephthalate glycol–based orthodontic template aligner material are summarized in Table 1 and illustrated in Figure 2.

Among the tested formulations, the highest fungal inhibition was recorded for MW-G, which achieved a fungal inhibition percentage (FIP) of 33.54%. This enhanced activity may be attributed to its essential oil–based composition, whose active components are known to exhibit antifungal properties and the ability to interfere with fungal membrane integrity and biofilm maturation.

In contrast, most of the other tested solutions demonstrated minimal or negligible antifungal activity, with inhibition values below 3%. Notably, MW-E showed a negative inhibition value (−13.21%), indicating a possible stimulatory effect on *C. albicans* biofilm development under the tested conditions.

Overall, the antifungal results highlight the limited efficacy of fluoride-based formulations when used alone against fungal biofilms on polymeric orthodontic surfaces, while essential oil–based solutions demonstrated superior performance in reducing fungal viability.

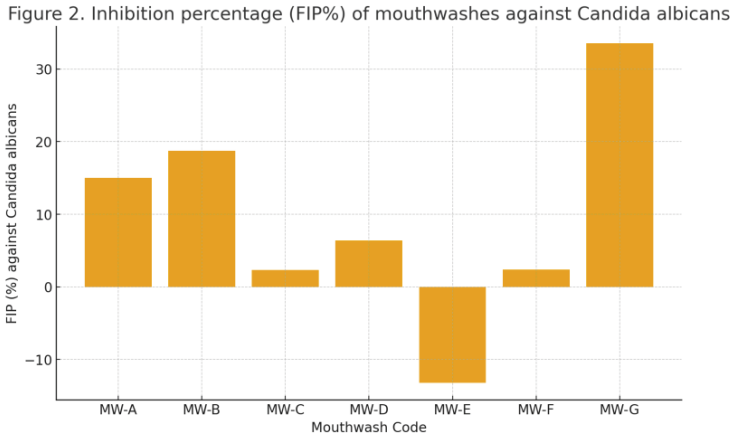

Figure 2. Inhibition percentages (FIP%) of the tested mouthwash solutions against Candida albicans biofilm developed on a polyethylene terephthalate glycol–based orthodontic template aligner material. Negative values indicate a possible stimulatory effect on fungal biofilm growth.

Comparative Antimicrobial Efficacy of Mouthwash Formulations

The overall antimicrobial performance was assessed by calculating the mean inhibition percentage across all three microbial strains.

The solution coded MW-B exhibited the highest overall efficacy, with a mean inhibition value of approximately 60.0%, followed by MW-G (46.4%). The remaining solutions demonstrated significantly lower overall activity, with MW-E presenting the poorest performance (mean BIP = −2.8%).

A moderate positive correlation was identified between the presence of fluoride in the formulation and the mean inhibition percentage (Spearman $\rho \approx 0.40$), indicating that fluoride contributes to antimicrobial efficacy but does not solely determine performance. In this context, essential oil–based formulations demonstrated notable efficiency despite the absence of fluoride.

A summary of the mean antimicrobial performance and fluoride content of the tested solutions is presented in Table 2.

Table 2. Fluoride concentration and mean antimicrobial inhibition of the tested mouthwash solutions.

Mouthwash Code	Fluoride Concentration (ppm F^-)	Mean BIP/FIP (%) across all strains
MW-A	0	16.8
MW-B	~225	60.0
MW-C	~100	21.9
MW-D	~250 (+ 0.05% CHX)	21.5
MW-E	0	−2.8
MW-F	~250	9.3
MW-G	0	46.4

Influence of the Aligner Template Material Surface

The polyethylene terephthalate glycol–based template aligner material showed a relatively smooth and hydrophobic surface, which may partially limit microbial adhesion. However, biofilm formation was still evident for all tested microorganisms.

The interaction between the polymer surface and the active compounds in the mouthwash solutions likely contributed to the observed differences in efficacy. Substances with higher substantivity on polymeric surfaces (such as chlorhexidine and certain essential oil compounds) showed prolonged antimicrobial effects compared to fluoride ions, which exhibit limited affinity for polymer substrates.

These surface–compound interactions may explain why some non-fluoridated solutions outperformed fluoridated ones in specific microbial contexts, particularly for *Candida albicans*.

DISCUSSIONS

This in vitro study evaluated the antimicrobial effects of seven commercially available mouthwash formulations on biofilms formed by Streptococcus mutans, Streptococcus oralis, and Candida albicans on a polyethylene terephthalate glycol–based orthodontic template aligner material. The results demonstrate that biofilm inhibition is strongly influenced by the chemical composition of the tested solutions, rather than solely by the presence or absence of fluoride [15].

Across all three microbial strains, the fluoride-containing antiseptic solution MW-B exhibited the highest overall antimicrobial efficacy, achieving inhibition percentages of 81.93% against S. mutans and 80.51% against S. oralis. This strong performance can be attributed to the combined presence of fluoride and cetylpyridinium chloride (CPC), a quaternary ammonium compound known for its membrane-disrupting antibacterial properties [16]. These findings support previous reports indicating that fluoride enhances

antimicrobial activity more efficiently when combined with additional antiseptic agents, rather than when used as a single active compound.

Interestingly, MW-G, a fluoride-free formulation based on essential oils, demonstrated high inhibitory activity against Candida albicans (33.54%) and good antibacterial performance against both streptococcal species. This confirms the antifungal and antibacterial potential of essential oil components such as eugenol, terpinen-4-ol, and cinnamaldehyde, which are known to alter microbial cell membrane permeability and disrupt enzymatic activity [9]. The superior antifungal performance of this formulation highlights the limited efficacy of fluoride alone against fungal biofilms and suggests that plant-derived bioactive compounds may represent valuable adjuncts in aligner hygiene protocols [17].

In contrast, MW-F, which contained amine fluoride, showed relatively low antimicrobial efficacy, despite its high fluoride concentration. This finding suggests that the chemical form of fluoride significantly influences its bioavailability and antimicrobial behavior on polymeric surfaces. While amine fluorides are effective for enamel remineralization due to their affinity for mineralized tissues, their antimicrobial performance on polymeric substrates such as orthodontic aligner materials may be limited, particularly in the absence of complementary antiseptic compounds [18] .

Of particular interest was the negative inhibition percentage observed for Candida albicans following exposure to MW-E, a plant-based formulation containing herbal extracts and low fluoride concentration. This phenomenon, also reported in the original Romanian study, may be explained by the presence of nutritive phytocompounds or carbohydrates that can transiently support fungal metabolism and biofilm development when present at subinhibitory concentrations [19] . Such effects have also been described in the literature, where certain botanical extracts act as prebiotic substrates for fungal species under specific conditions [20].

The moderate positive correlation identified between fluoride presence and mean inhibition percentage (Spearman's $\rho \approx 0.40$) further supports the hypothesis that fluoride contributes to antimicrobial activity but does not solely determine it. Instead, the overall formulation—including antiseptic agents, essential oils, alcohol, and excipients—plays a central role in modulating antimicrobial performance on polymer-based orthodontic materials [21].

From a clinical perspective, these findings are particularly relevant for orthodontists using template aligners during attachment bonding procedures. Although these materials are usually employed for short-term intraoral contact, their close adaptation to tooth surfaces and frequent handling make them potential vectors for microbial contamination. The results of this study indicate that not all mouthwashes provide equal decontamination efficacy on template aligner surfaces and that essential oil–based or CPC-containing formulations may offer superior antimicrobial benefits compared to fluoride-only rinses.

Nevertheless, this study has several limitations. It was conducted under in vitro conditions, using mono-species biofilms and a single exposure time of one minute, which may not fully replicate the complexity of the oral environment. In clinical reality, aligners and templates are exposed to mixed microbial communities, saliva proteins, and dynamic forces that may influence biofilm adhesion and disinfectant efficacy. Additionally, the absence of a chemical neutralizer after mouthwash exposure may have led to a slight overestimation of antimicrobial effects; however, since all specimens were treated uniformly, the comparative integrity of the results remains valid.

Future studies should explore multispecies biofilm models, repeated disinfection cycles, and longer exposure protocols to better simulate clinical conditions. Furthermore, evaluating the surface morphology and physicochemical changes of template aligner

materials after repeated exposure to different disinfectant agents would provide valuable insight into the long-term implications for clinical usability and aesthetic stability [16].

CONCLUSIONS

This in vitro study demonstrated that the antimicrobial efficacy of mouthwash solutions on biofilms formed on a polyethylene terephthalate glycol–based orthodontic template aligner material depends primarily on their overall formulation rather than on fluoride concentration alone. The mouthwash containing fluoride and cetylpyridinium chloride showed the strongest antibacterial activity, while the essential oil–based solution proved most effective against Candida albicans. In contrast, some fluoride-only formulations exhibited limited antimicrobial effects. These findings suggest that essential oil–based and combination antiseptic mouthwashes may represent more suitable options for reducing microbial contamination of orthodontic template aligner materials in clinical practice. Further research using multispecies biofilm models and repeated exposure protocols is needed to better simulate clinical conditions.

Acknowledgments

We would like to acknowledge Victor Babes University of Medicine and Pharmacy, Timisoara, for their support in covering the costs of publication for this research paper. The authors are truly grateful to the staff of the Faculty of Dental Medicine, Victor Babes University of Medicine and Pharmacy, Timisoara, Romania, and to the Faculty of Food Engineering, University of Life Sciences "King Mihai I", Timisoara, Romania, for their valuable support. The authors used ChatGPT, an AI language model developed by OpenAI, exclusively to improve the manuscript's language and readability. All the scientific content, interpretations, and conclusions are the original work of the authors.

Conflicts of Interest

The authors declare no conflicts of interest.

REFERENCES

[1] Kiatwarawut K, Rokaya D, Sirisoontorn I. Antimicrobial Activity of Various Disinfectants to Clean Thermoplastic Polymeric Appliances in Orthodontics. Polymers. 2022 May 31;14(11):2256.

[2] Charavet C, Gourdain Z, Graveline L, Lupi L. Cleaning and Disinfection Protocols for Clear Orthodontic Aligners: A Systematic Review. Healthcare. 2022 Feb 10;10(2):340.

[3] Lucchese A, Marcolina M, Mancini N, Ferrarese R, Acconciaioco S, Gherlone E, et al. A comparison of the alterations of oral microbiome with fixed orthodontic therapy and clear aligners: a systematic review. Journal of Oral Microbiology. 2025 Dec 31;17(1):2372751.

[4] Belanche Monterde A, Flores-Fraile J, Pérez Pevida E, Zubizarreta-Macho Á. Biofilm Composition Changes During Orthodontic Clear Aligners Compared to Multibracket Appliances: A Systematic Review. Microorganisms. 2025 Apr 30;13(5):1039.

[5] Hamdoon SM, AlSamak S, Ahmed MK, Gasgoos S. Evaluation of biofilm formation on different clear orthodontic retainer materials. J Orthod Sci. 2022;11:34.

[6] Pasaoglu Bozkurt A, Demirci M, Erdogan P, Kayalar E. Comparison of microbial adhesion and biofilm formation on different orthodontic aligners. American Journal of Orthodontics and Dentofacial Orthopedics. 2025 Jan;167(1):47–62.

[7] 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. Dentistry Journal. 2023 Jan 3;11(1):13.

[8] Kiatwarawut K, Kuvatanasuchati J, Thaweboon B, Sirisoontorn I. Comparison of Various Antimicrobial Agents for Thermoplastic Polymeric Retainers. Polymers. 2022 Sep 8;14(18):3753.
[9] Alqarni H, Ba-Armah I, Almutairi N, Alenizy M, Arola DD, Oates TW, et al. Novel antimicrobial and bioactive resin-based clear aligner attachment orthodontic materials. Front Oral Health. 2025 Aug 7;6:1630019.
[10] 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 Periodontics Orthod. 2025;5(1):33–42.
[11] Astasov-Frauenhoffer M, Göldi L, Rohr N, Worreth S, Dard E, Hünerfauth S, et al. Antimicrobial and mechanical assessment of cellulose-based thermoformable material for invisible dental braces with natural essential oils protecting from biofilm formation. Sci Rep. 2023 Aug 18;13(1):13428.
[12] Harzivartyan S, Yilmaz HN, Nalbantoglu ER, Topcuoglu N. Evaluation of Microbial Colonisation on Clear Aligners With Different Cleaning Methods: A Prospective In Vivo Cross-Over Study. Orthod Craniofacial Res. 2025 Sep 25;ocr.70039.
[13] Esmaeili S, Shahbazi S, Ziaei H, Younessian F. Evaluating the Impact of Clear Dental Aligners on Dental and Periodontal Conditions, Oral Microbial Patterns, and the Immune System. In: Rezaei N, Ziaei H, editors. Oral Immunology [Internet]. Cham: Springer Nature Switzerland; 2026 [cited 2025 Nov 25]. p. 605–24. (Advances in Experimental Medicine and Biology; vol. 1492). Available from: https://link.springer.com/10.1007/978-3-032-03176-1_29
[14] Badnjević M, Petković Didović M, Jelovica Badovinac I, Lučić Blagojević S, Perčić M, Špalj S, et al. Disruption of Early Streptococcus mutans Biofilm Development on Orthodontic Aligner Materials. Processes. 2025 Sep 25;13(10):3069.
[15] Latimer J, Munday JL, Buzza KM, Forbes S, Sreenivasan PK, McBain AJ. Antibacterial and anti-biofilm activity of mouthrinses containing cetylpyridinium chloride and sodium fluoride. BMC Microbiol. 2015 Aug 21;15:169.
[16] Arce-Toribio JP, Arbildo Vega HI, López-Flores AI. Antimicrobial Efficacy of the Use of Mouthwash with Cetylpiridinium Chloride for Aerosol Producing Procedures. A Systematic Review. Odovtos - Int J Dent Sc. 2023 May 8;10–23.
[17] Valdivia-Tapia AC, Lippert F, Gregory RL. Evaluation of the antimicrobial potential of fluoride-free mouthwashes against Scardovia wiggsiae. Eur Arch Paediatr Dent [Internet]. 2025 Oct 8 [cited 2025 Nov 25]; Available from: https://link.springer.com/10.1007/s40368-025-01116-4
[18] Brookes ZLS, Bescos R, Belfield LA, Ali K, Roberts A. Current uses of chlorhexidine for management of oral disease: a narrative review. Journal of Dentistry. 2020 Dec;103:103497.
[19] Takenaka S, Sotozono M, Ohkura N, Noiri Y. Evidence on the Use of Mouthwash for the Control of Supragingival Biofilm and Its Potential Adverse Effects. Antibiotics. 2022 May 28;11(6):727.
[20] Gaaloul H, Amor WB, Dhia RB, Haddad O, Tobji S, Mastouri M, et al. Qualitative Modifications of the Aligners' Bacterial Flora under the Effect of Tea Tree Essential Oil. Saudi J Oral Dent Res. 2022 Apr 16;7(4):107–15.
[21] Bichu YM, Alwafi A, Liu X, Andrews J, Ludwig B, Bichu AY, et al. Advances in orthodontic clear aligner materials. Bioactive Materials. 2023 Apr;22:384–403.