

ORTHODONTIC APPLIANCES AND MICROBIAL DYSBIOSIS: INSIGHTS AND IMPLICATIONS

Abstract

Orthodontic treatment can lead to significant changes in the oral microbiome, potentially resulting in dysbiosis associated with increased risk of gingivitis and caries. The complex hard-soft tissue structures in the mouth harbour a diverse bacterial community, with around 700 different species identified. Orthodontic appliances, such as fixed braces, can alter the oral environment, leading to changes in the composition and abundance of oral microorganisms.

Studies have shown that fixed orthodontic appliances, including brackets and bands, can influence the colonization of periodontal pathogens like *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum*, which are associated with periodontal diseases. These appliances can also increase the levels of cariogenic bacteria such as *Streptococcus mutans* and *Lactobacilli*, which are linked to dental caries.

On the other hand, clear aligners have been found to have a lesser impact on the oral microbiome compared to traditional braces, with some studies suggesting that they may even reduce the negative effects on periodontal health. However, clear aligners can still lead to changes in the oral microbiome, including increases in bacteria associated with gingivitis and periodontitis.

Overall, orthodontic appliances can significantly alter the oral microbiome, potentially leading to dysbiosis and increased risk of oral diseases. Proper oral hygiene instructions and monitoring are essential to minimize these risks during orthodontic treatment.

Keywords : *Fixed Orthodontic Appliances, Removable Orthodontic Appliances, Cariogenic Bacterias, Periodontal Pathogens.*

INTRODUCTION

The mouth is home to around 700 distinct species of bacteria, making it one of the most diverse bacterial communities in the human body. The mouth has complex hard-soft tissue structures, such as teeth, the tongue, the gingiva, and the palate; depending on the different surface properties, distinct differences in the oral microbiota's structure can be seen.[23]

Prokaryotes from about 700 different species have been found in the mouth cavity. Approximately 54% of these species have official names, 32% of the species are solely recognised as uncultivated phylotypes, while 14% are unnamed but farmed. These species belong to 12 phyla and 185 genera. There are 12 different phyla of bacteria: Firmicutes, Fusobacteria, Proteobacteria, Actinobacteria, Bacteroidetes, Chlamydia, Chloroflexi, Spirochaetes, SR1(Absconditabacteria), Synergistetes, Saccharibacteria (TM7), and Gracilibacteria (GN02).[76]

FIXED ORTHODONTIC APPLIANCE

PERIODONTAL PATHOGENS

Gingivitis (and periodontitis) development is influenced by a number of different factors. It has recently been demonstrated that bracket designs and material properties can affect the clinical parameters and bacterial profile.[4,5] The structure of orthodontic bands is complicated. They are made up of numerous stainless-steel parts that are joined together either by soldering or welding, and local irritation may be brought on by corrosion products and their effects on biofilms. [6-8]

In one clinical research, individuals with chronic periodontitis receiving fixed orthodontic therapy experienced a significant decrease in the overall number of bacteria in the pocket; this could have been due to the materials employed, which altered the environment for biofilm growth. The placement of orthodontic appliances had an impact on clinical indicators and the colonisation of periodontal bacteria that are pathogenic, such as *P. gingivalis*, *P. intermedia*, *P. nigrescens*, and *F. nucleatum*. [1,17-18]

In order to examine and contrast the oral microflora between orthodontic recipients and individuals in good health, Fubo Sun et al. used amplification of the 16S rRNA V3 region, analysis by PCR-DGGE, and quantifying of dominant species by real-time quantitative PCR.[10]. The majority of the amplified bands that were chosen, eliminated, and sequenced for taxonomic identification belonged to Firmicutes and proteobacteria. Each group had *Streptococcus* and *Neisseria* species, but only those receiving orthodontic treatment had *Pseudomonas*, *Veillonella*, and *Burkholderia* species. This was consistent with another investigation that found opportunistic *Pseudomonas* species in orthodontic recipients and attributed this to *Pseudomonas aeruginosa*'s superior ability to cling to dental surfaces over *Streptococcus pneumoniae*. [11]. According to a recent study, orthodontic patients had considerably higher levels of *Pseudomonas* species in addition to higher levels of coliforms such *Enterobacter*, *Acinetobacter*, and *Yersinia*. [16]

Additionally, Slots et al. identified *Pseudomonas* bacteria in the subgingival microflora of individuals with advanced adult periodontitis.[12]. Patients with orthodontic issues have also had opportunistic infections such *Veillonella*, *Neisseria*, and others. According to Kim et al and Moon et al [13,14], *Veillonella* has also been discovered in a plaque that present subgingivally of the of Korean patients who cigarette smoke and have severe periodontitis, while *Burkholderia* spp. have been discovered in samples of dentine caries.[15]

Proteobacteria make up a large portion of the typical flora found in the gut and mouth cavity, whereas Actinobacteria play a significant role in the ambient microbiome. Actinobacteria significantly decreased with the use of fixed orthodontic appliances, according to earlier research[19]. According to certain research, saccharibacteria rise with ageing and have a part in the development of periodontitis[21,22].

The number of organisms from the genus *Actinobacillus*, *Actinomyces*, *Corynebacterium*, *Kingella*, and *Neisseria* as well as the species *Haemophilus parainfluenzae*, *Lautropia mirabilis*, and *Rothia dentocariosa* decreased noticeably in plaque samples.[23] According to several research, orthodontic treatment significantly increases the plaque level of *Actinomyces*.

After the appliances were taken out, *Actinomyces naeslundii* were more , according to Koopman et al.'s research on the genus *Actinomyces*. According to Tanner et al., *Actinomyces* is connected to gingivitis brought on by orthodontic therapy.[25] *Neisseria* has been associated with a group of orthodontic patients who have mild gingivitis, and it has been observed that its prevalence increases twelve weeks after removal and declines as removal approaches. [19,25].

Three months after the initiation of orthodontic therapy, *T. forsythia* showed a sizable increase, according to the systematic analysis conducted by Gou et al., yet the information was mainly concerned with plaque that is subgingivally.[27] The results of Isamu Kado's thorough examination and inquiry strongly suggested that the usage of appliances for orthodontics increased the amount of the genus *Tannerella* in the oral cavity.

According to Zhao et al., despite employing the NGS (Next Generation Sequencing) approach, there was a significant decline in Prevotella abundance and no change in the composition of the overall microbial community in saliva. Patients with orthodontic treatment who had plaque at white-spot lesions showed a considerably higher detection rate for Granulicatella elegans. [25], in contrast to research by Tanner et al, conducted by PCR, which stated the saliva notably included less of the genus Granulicatella.

These changes indicate that facultative anaerobic and aerobic microbes were replaced in the oral microbiota, namely in plaque, following the placement of fixed orthodontic devices, by obligate anaerobes and periodontopathogenic bacteria.

CARIOGENIC BACTERIA

Since *S. mutans* and *Lactobacillus* are important pathogenic bacteria, O'Reilly reported that demineralization was seen a month after bonding in WSLs and dental caries. *S. mutans* and *Lactobacillus*, however, have been shown to significantly increase from month three to month six of orthodontic therapy, according to Topaloglu and Chang et al.[32,33] However, Kupietzky et al. and Jurela A, et al. have shown that there was no discernible difference between the levels of *S. mutans* and other bacteria within the first three months.[34,35] However, the bulk of WSL or dental caries observations have been made after 2 years of orthodontic therapy, therefore bacterial modifications may have gone unnoticed in studies that lasted for a shorter period of time.

Over the course of the 18-month treatment, *S. mutans* prevalence increased considerably only in patients wearing conventional braces, while the proportion of *S. mutans* in the SLB group stayed at a significantly lower level with no change. Pellegrini et al. and Akin et al. both reported the same findings in their studies.[37,38]

According to Jing et al.'s studies, lactobacillus levels somewhat increased, which is in line with Peros and Lara-Carrillo et al.'s findings. In a systematic review study, Lucchese et al.[39,40] noted that the use of orthodontic appliances had an impact on the increase in numbers of *Lactobacillus* and *S. mutans*. [41]

A cross-sectional investigation, Klaus et al. found that poor oral hygiene was substantially associated with a greater incidence of *Candida* spp. in plaque and saliva. The two primary species discovered were *Candida albicans* and *Candida dubliniensis*. In 100% of salivary samples and 91% of plaque samples, respectively, *S. mutans* and *Lactobacilli* were both found to be bacteria.[42]. Topaloglu-Ak et al. carried out a comparable investigation on the use of fixed and detachable devices for the cultured identification of salivary *S. mutans*, *Lactobacillus* spp., and *C. albicans*. Six months following the installation of fixed/removable appliances, they discovered a statistically significant rise in *S. mutans* and *Lactobacilli*, as well as a higher presence of *C. albicans* in the fixed appliance group than in the detachable appliance group.[43]

Andrucioli et al. [44] examined bacterial contaminants following 30 days of premolar bands left in place following 16 months of fixed orthodontic therapy by utilising checkerboard DNA-DNA hybridization. *S. mutans* and *Streptococcus sobrinus* were more prevalent among the cariogenic species compared to *Lactobacillus acidophilus* and *Lactobacillus casei*.

According to Maria et al.'s study, there was no discernible change in the species of streptococcal bacteria between the orthodontic and healthy groups.[45]

SELF LIGATING BRACKET

According to research by Peter et al. [2], as compared to Elastomer appliances, Self-ligating appliances promote decreased oral microbial retention, including streptococci. The majority of patients' teeth with SL attachments displayed a reduced amount of ATP bioluminescence and fewer bacteria in plaque than teeth attached to brackets with elastomer, Jing et al. [7] found a substantial rise in *S. mutans* in individuals with traditional brackets.

When compared to conventional brackets ligated with stainless steel ligatures, Baka et al. and Uzener et al. did not find any statistically significant differences, but they did discover an increase in gram-negative and gram-positive bacteria (mainly Streptococci and Lactobacilli).[8.9]

CERAMIC BRACKET

The amounts of *P. nigrescens*, *Actinomyces odontolyticus*, *T. forsythia*, *Actinomyces naeslundii*, *Capnocytophaga ochracea*, *Actinomyces israelii*, and cariogenic bacteria like *S. mutans* and *L. acidophilus* were also very similar on metallic and ceramic brackets isolated from both front and back teeth, according to Anhoury et al.

Most significantly increasing species in ceramic ones were *Selenomonas noxia*, *Capnocytophaga showae*, and *E. corrodens*. *Actinomyces gerencseriae*, *Streptococcus constellatus*, and *Streptococcus sanguis* counts all considerably increased in anterior ceramic brackets

LIGATURE WIRE VS ELASTOMERIC MODULE

In comparison to incisors ligated with steel wires, archwires with elastomeric rings contained more microbes, according to research by Forsberg et al, [1] examined that how microbial plaque retention around fixed appliances were ligated, with steel ligatures and elastomeric ties affected the area.

After conducting a split-mouth investigation, Türk kahraman et al.[5] found that elastomeric rings caused greater bleeding at the teeth than steel ligatures did. *T. forsythia* and *P. nigrescens* were found in significantly larger concentrations at elastomeric ligatures, according to Alves de Souza et al. [6], but there were no appreciable differences between *P. gingivalis*, *A. actinomycetemcomitans*, and *P. intermedia*.

MOLAR BANDS AND TUBES

With the aid of 16S rDNA microarray and denaturing gradient gel electrophoresis (DGGE), Ireland et al. [3] discovered that *T. denticola* and *P. nigrescens* were on the rise while *A. actinomycetemcomitans* was on the decline. Plaque connected to both types of molar attachments had higher concentrations of *P. gingivalis*, *T. forsythia*, and *E. nodatum*, but interestingly only bonded molars were used to produce *C. rectus*, *Parvimonas micra*, *A. odontolyticus*, and *V. parvula*.

Mártha et al.'s DNA-strip method [4] was used to determine if subgingival plaque contained periodontopathogen bacteria. *F. nucleatum* was the most prevalent bacterial species across all groups and periods. Then, *Capnocytophaga* species (*C. gingivalis*, *C. ochracea*, and *C. sputigena*) and *E. corrodens*.

LABIAL VS LINGUAL

In their comparative investigation between the biofilm formation on the labial and lingual bracket surfaces, Yener et al. discovered that the biofilm accumulation on the lingual orthodontic therapy surface was more than that on the labial orthodontic therapy surface. For labial and lingual brackets, the locations with the largest biofilm buildup are the gingival, mesial, and distal surfaces [81]. On the

other hand, Sfondrin et al. did not discover any appreciable variations in clinical periodontal markers or microbiological results between buccal and lingual brackets[82].

Using the checkerboard DNA-DNA hybridisation approach, Gujar et al. assessed and compared the degree of appearance of orange and red microbial complexes in individuals receiving orthodontic treatment with aligners, traditional metallic fixed labial appliances, and lingual fixed appliances. They discovered that the lingual appliance had greater percentages of *T. denticola*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum*. The study found that lingual fixed appliances exhibited greater microbiological contamination than labial fixed appliances, then with aligners. *Fusobacterium periodontium* and *Prevotella intermedia* were found in higher percentages in the labial fixed appliance [83]. According to Demling et al.'s investigation, the relative prevalence of Aa and Pg did not alter when fixed lingual appliances were inserted without supportive dental prophylaxis, although clinical characteristics specific to the lingual sites worsened [84].

REMOVABLE ORTHODONTIC APPLIANCE

Zharmagambetova et al. evaluated the effects of orthodontic treatment with ROA on 12-year-old individuals with dentoalveolar abnormalities in the oral microbiota. They discovered that *C. albicans*, *S. aureus*, and *S. mutans* frequency increased and the normal level of the microbiota reduced.[46]

According to research by Arendorf et al., there is a clear connection between the usage of ROA and the occurrence of *Candida*. [47]

After employing the ROA for 4 weeks, Marisela et al. revealed that the ROA and the supporting oral mucosa contained *S. aureus*, *P. aeruginosa*, and *Candida* spp. *S. aureus* was the most abundant bacterium in both the supporting oral mucosa and the ROA. This bacterium has a significant death rate and is connected to respiratory tract illnesses. *P. aeruginosa* is one of the most important lung infections and the leading cause of mortality as well as morbidity in cystic fibrosis patients. The results revealed that *P. aeruginosa* was the second-highest prevalent bacterium in the supporting oral mucosa and the ROA.

Additionally, Batoni et al. discovered that children receiving removable orthodontic appliances had a higher number of mutans streptococci.[50] According to Sumi et al., acrylic bases can increase the risk of pharyngeal colonisation and aspiration pneumonia by acting as a reservoir for respiratory infections.[51]

A potential direct linear association between the existence of ROAs, *C. albicans*, and salivary PH levels was declared by prior investigations.[54,55] Although *C. Albicans* levels significantly increased as a result of ROAs, *C. Dublinensis* levels did not statistically significantly rise, according to Farrokh et al. [53] This outcome is consistent with numerous studies that found *C. albicans* yeast to be highly and significantly prevalent in orthodontic patients.

Full-length 16S rRNA gene sequencing was employed in a study by Fernanda et al. to examine biofilm adhering to acrylic retainers on platforms created by ONT (Oxford Nanopore Technologies) for the V1-V9. In every sample they found, the phyla Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria were highly represented. Six phyla were identified in this study: the Firmicutes (*Streptococcus*, *Gemella*, *Eubacterium*, *Selenomonas*, *Veillonella* and related ones), the Actinobacteria (*Actinomyces*, *Atopobium*, *Rothia*, etc.), the Proteobacteria (*Neisseria*, *Eikenella*, *Campylobacter* and related ones), the Bacteroidetes (*Capnocytophaga*, *Porphyromonas*, *Prevotella*, etc.), the Fusobacteria (*Leptotrichia*

and Fusobacterium) and the phylum of TM7. The most firmicute phyla are found on the plaque on the retainer. When there are a lot of Firmicutes in the mouth, polysaccharide hydrolysis has started. Furthermore, Firmicutes are also very important in the connection between gut bacteria and health. Firmicutes play a critical role in the capacity of the human body to take in fats and break down lipids, which is essential to staying healthy. The most common genus found clinging to acrylic retainers was likewise found to be Streptococcus. Actinomyces has been found to be the most prevalent bacterial species associated with dentures, contrary to earlier studies by Shi et al.[58]

In their report on pathogenic bacteria, they also mentioned Propionibacterium propionicum, Gordonia bronchialis, Campylobacter gracilis, Campylobacter nucleatum, Prevotella loescheii, Capnocytophaga granulosa, and other Porphyromonas species.[58]

According to Alessandra et al.'s systematic review, Candida colonies grow during the first month of treatment, particularly those of the C. albicans species, and then they start to decline after a few months. The major rise in S. mutans occurs in the first 15 days of treatment and continues over the first few months. The microbiological count of Lactobacillus spp. rises over the first few months of treatment. During the first month of treatment, Moraxella catharralis and S. epidermidis levels considerably rise. During the first 6-7 months of treatment, Spirochaetes spp. considerably rises. Prevotella nigrescens, Aa, Pg, and Tf were not found following treatment.[59]

CLEAR ALIGNERS

Zhao et al. used 16S rRNA to examine the microecology of saliva and found that the use of clear aligners had no discernible effect on the microbial diversity of saliva.[60]

In their work, Dong et al. discovered that over varying lengths of aligner usage, the diversity and constitution of the microbiome underwent significant changes at the phylum, order, genus, and species levels. There had been more Firmicutes than at the onset of the treatment at the phylum level. After 24 hours, Lactobacillales and Bacteroidales were abundant at the order level. Actinomycetales were first abundant in large numbers but then substantially declined. Streptococcus, Haemophilus, and Porphyromonas had higher genus-level abundances from T0 to T24 h, whereas Rothia, Lautropia, and Actinomyces had lower abundances. Streptococcus infantis' species-level abundance increased after 24 hours, but that of Streptococcus anginosus and Rothia dentocariosa more in comparison to initial stage.[61]

However, as aligner usage length increased, Actinomyces abundance dropped. This may be because the inside surface of aligners did not provide favourable environment for colonisation or because the observation period used in this study was too short to allow for microbial growth. [62]

Also In this study, Streptococcus and Lactobacillus, two key microbiome components associated with acid production and tooth caries, rose in abundance between 0 and 24 hours. The findings of this study reveal that as aligner usage length increases, the stability of the core microbiota declines, indicating the possibility of an unhealthy environment developing on the inner surface of aligners.

Despite the fact that patients receiving CAT had their teeth and gingiva covered for almost the entire day, Miethke et al. found that periodontal risk was much lower in those receiving the treatment. [63] The use of clear aligners may lessen the negative effects of orthodontic treatments on periodontal health, according to a study by Rossini et al. [64]

According to Kabilan et al.[65], the microbial community in saliva fluctuated between days 7 and 14, with the abundance of certain taxa altering considerably between days 7 and 14. The clear retainer held the most numbers of Streptococcus species from the Firmicutes phylum at both 7 and 14 days. This findings by Yan et al. are supported by data.[61] In the investigation by Kabilan et al., compared to saliva, Granulicatella increased by almost 2 log at day 7. However, in a different study by Tanner et

al[25], *Granulicatella elegans* was found in plaque at a much higher level in a group of orthodontic patients with white-spot lesions.

The amount of parascardovia, which is generally isolated from dental caries, was significantly higher in the retainer as compared to saliva at 7 days. A gram-positive, functionally anaerobic cocci called *Gemella* has the potential to induce infective endocarditis; at all time intervals, bacteremia was also markedly elevated in the clear retention. *Actinomyces* decreased with rising retainer usage, mirroring the findings of Yan et al. This might be the case because *Actinomyces* would not thrive in this environment.

Additionally, the largest rates of Firmicutes, Bacteroidota, Proteobacteria, Actinobacteriota, and Fusobacteriota were found in the samples, which is similar with Kado et al.'s [23] findings. They also found that Actinobacteria levels on the retainer and in the saliva were consistent from 7 to 14 days, while the latter's levels were marginally higher than those on the clear retainer.

At 7 and 14 days, genus-level findings of gingivitis-related bacteria, including *Solobacterium*, *Parvimonas*, and *Selenomonas*, in the supragingival plaque, were also made in the retainer. At both 7 and 14 days, the retainer biofilm contained *Tannerella* and *Fusobacterium*, which have the potential to induce periodontitis. [65]

According to research by Qian et al. [66], the Invisalign group was shown to have a lower abundance of Firmicutes than the fixed appliance group while being similar to the control group. This finding suggested that the Invisalign group was more like a control group. According to the findings, *Neisseria* was more common at the genus level in the fixed appliance group than the Invisalign group. *Neisseria* colonises tooth surfaces quickly, and some studies have found a link between *Neisseria* and better dental health or less gingivitis. [25]

RETAINERS

After orthodontic treatment with fixed appliances, Eroglu et al. compared and evaluated salivary microbial levels and periodontal status in patients using a fixed lingual retainer, a removable vacuum-formed retainer, or a Hawley retainer. They discovered that salivary *S mutans* and *L casei* levels and periodontal status do not differ between fixed and removable orthodontic retainers [77]. In addition, Bowen et al. compared three different types of retainers and discovered that, after six months of usage, the Hawley retainer outperformed the vacuum-formed retainer and the fixed lingual retainer in terms of Pg and Aa content and periodontal clinical characteristics [78].

Fixed retainers may increase cariogenic and periodontal infections and damage oral health, according to a study by Dhuha et al, A substantial rise in *Streptococcus mutans*, *Lactobacillus acidophilus*, *Aggratibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Candida albicans* was observed [79]. According to Kabilan et al's study, wearing a transparent retainer may cause alterations to the enamel or damage of periodontal tissue, particularly after 14 days of use [80].

TREATMENT STRATEGIES AND CLINICAL IMPLICATION

Using various orthodontic appliances can alter or enhance the oral microbiota, which increases the risk of developing periodontal diseases, caries, and white spot lesions. In the brief time after orthodontic appliance placement, it has been discovered that caries-causing bacteria such as *Streptococcus* and *Lactobacillus* are proliferating. Patients receiving orthodontic treatment are more likely to get halitosis if certain bacteria are growing. All of these raise the possibility that a patient may not be practicing proper dental hygiene. Treatment methods to avoid these include encouraging and educating patients about maintaining good hygiene, teaching them how to brush and floss properly, prescribing fluoride mouthwash for patients who are at risk of dental caries, using proper bonding and banding techniques and removing excess bonding material from the edges of braces and bands, and providing instructions on how to clean and take care of removable orthodontic appliances. Punnisa et al. found that

combination therapy, which included brushing and Polident Pro Guard & Retainer®, was the most efficient way to remove retainer biofilms. Furthermore, because Polident Pro Guard & Retainer® did not change the homeostatic balance of the bacterial populations attached to the acrylic retainers, it was safe to use.

CONCLUSION

Orthodontic treatment causes profound alterations in the oral bacterial environment that are linked to gingivitis and a higher risk of cariogenic responses. Giving patients oral hygiene instructions and monitoring their oral hygiene both before and during therapy are very important.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

REFERENCES

1. Naranjo AA, Trivino ML, Jaramillo A, Betancourth M, Botero JE. Changes in the subgingival microbiota and periodontal parameters before and 3 months after bracket placement. *Am J Orthod Dentofacial Orthop* 2006;130:275.e17-22
2. Clinical and microbiological findings at sites treated with orthodontic fixed appliances in adolescents Klara Kim,^a Kristin Heimisdottir,^b Urs Gebauer,^c and G. Rutger Persson^d. 2010 by the American Association of Orthodontists.
3. Marsh PD, Martin MV. *Oral microbiology*. Stuttgart: Georg Thieme Verlag; 2003. p. 20-37
4. Gastel J, Quirynen M, Teughels W, Coucke W, Carels C. Influence of bracket design on microbial and periodontal parameters in vivo. *J Clin Periodontol* 2007;34:423-31.
5. Anhoury P, Nathanson D, Hughes CV, Socransky S, Feres M, Chou LL. Microbial profile on metallic and ceramic bracket materials. *Angle Orthod* 2002;72:338-43.)
6. Grimsdottir MR, Gjerdet NR, Hensten-Pettersen A. Composition and in vitro corrosion of orthodontic appliances. *Am J Orthod Dentofacial Orthop* 1992;101:525-32.
7. Grimsdottir MR, Hensten-Pettersen A, Kullmann A. Cytotoxic effect of orthodontic appliances. *Eur J Orthod* 1992;14:47-53.
8. Grimsdottir MR, Hensten-Pettersen A. Cytotoxic and antibacterial effects of orthodontic appliances. *Scand J Dent Res* 1993; 101:229-31
9. Speer C, Pelz K, Hopfenmüller W, Holtgrave EA. Investigations on the influencing of the subgingival microflora in chronic periodontitis. A study in adult patients during fixed appliance therapy. *J Orofac Orthop* 2004;65:34-47.)
10. Comparison of Oral Microbiota in Orthodontic Patients and Healthy Individuals Fubo Sun¹ , Ayaz Ahmed², Lina Wang³ , Ming Dong⁴ , Weidong Niu³ * , *Microbial Pathogenesis* (2018),journal of micropathology
11. Kitada K, de Toledo A, Oho T: Increase in detectable opportunistic bacteria in the oral cavity of orthodontic patients. *Int J Dent Hyg* 2009
12. Slots J, Rams TE, Listgarten MA: Yeasts, enteric rods and pseudomonads in the subgingival flora of severe adult periodontitis. *Oral Microbiol Immunol* 1988,

13. Kim K, Heimisdottir K, Gebauer U, Persson GR: Clinical and microbiological findings at sites treated with orthodontic fixed appliances in adolescents. *Am J Orthod Dentofacial Orthop* 2010, 137(2):223-228
14. Moon JH, Lee JH, Lee JY: Subgingival microbiome in smokers and non-smokers in Korean chronic periodontitis patients. *Mol Oral Microbiol* 2015
15. Goh SY, Tan WS, Khan SA, Chew HP, Abu Kasim NH, Yin WF, Chan KG: Unusual multiple production of N-acylhomoserine lactones a by Burkholderia sp. strain C10B isolated from dentine caries. *Sensors (Basel)* 2014
16. Hagg U, Kaveewatcharanont P, Samaranayake YH, Samaranayake LP: The effect of fixed orthodontic appliances on the oral carriage of Candida species and Enterobacteriaceae. *Eur J Orthod* 2004
17. Ristic, M., Vlahovic Svabic, M., Sasic, M. & Zelic, O. Clinical and microbiological effects of fixed orthodontic appliances on periodontal tissues in adolescents. *Orthod. Craniofac. Res.* 10, 187–195. <https://doi.org/10.1111/j.1601-6343.2007.00396.x> (2007).
18. Goyal, N. et al. Effects of amine fluoride and probiotic mouthwash on levels of Porphyromonas gingivalis in orthodontic patients: A randomized controlled trial. *J. Indian Soc. Periodontol.* 23, 339–344. https://doi.org/10.4103/jisp.jisp_551_18 (2019)
19. Koopman, J. E. et al. The effect of fixed orthodontic appliances and fluoride mouthwash on the oral microbiome of adolescents—A randomized controlled clinical trial. *PLoS ONE* 10, e0137318. <https://doi.org/10.1371/journal.pone.0137318> (2015)
20. Ristic, M., Vlahovic Svabic, M., Sasic, M. & Zelic, O. Clinical and microbiological effects of fixed orthodontic appliances on periodontal tissues in adolescents. *Orthod. Craniofac. Res.* 10, 187–195. <https://doi.org/10.1111/j.1601-6343.2007.00396.x> (2007).
21. Crielaard, W. et al. Exploring the oral microbiota of children at various developmental stages of their dentition in the relation to their oral health. *BMC Med. Genom.* 4, 22. <https://doi.org/10.1186/1755-8794-4-22> (2011).
22. Duran-Pinedo, A. E. et al. Community-wide transcriptome of the oral microbiome in subjects with and without periodontitis. *ISME J.* 8, 1659–1672. <https://doi.org/10.1038/ismej.2014.23> (2014).
23. The impact of fixed orthodontic appliances on oral microbiome dynamics in Japanese patients Isamu Kado^{1,2}, Junzo Hisatsune^{2,4}, Keiko Tsuruda^{2,3}, Kotaro Tanimoto¹ & Motoyuki Sugai^{2,4}, *Scientific Reports* | (2020) 10:21989 | <https://doi.org/10.1038/s41598-020-78971-2>
24. Nadkarni, M. A. et al. Pattern of distribution of Prevotella species/phylotypes associated with healthy gingiva and periodontal disease. *Eur. J. Clin. Microbiol. Infect. Dis.* 31, 2989–2999. <https://doi.org/10.1007/s10096-012-1651-5> (2012).
25. Tanner, A. C. et al. White-spot lesions and gingivitis microbiotas in orthodontic patients. *J. Dent. Res.* 91, 853–858. <https://doi.org/10.1177/0022034512455031> (2012).
26. Choi, D. S. et al. Microbiologic changes in subgingival plaque after removal of fixed orthodontic appliances. *Angle Orthod.* 79, 1149–1155. <https://doi.org/10.2319/111808-593r.1> (2009).
27. Guo, R., Lin, Y., Zheng, Y. & Li, W. The microbial changes in subgingival plaques of orthodontic patients: A systematic review and meta-analysis of clinical trials. *BMC Oral Health* 17, 90. <https://doi.org/10.1186/s12903-017-0378-1> (2017).
28. Mysak, J. et al. Porphyromonas gingivalis: Major periodontopathic pathogen overview. *J. Immunol. Res.* 2014, 476068. <https://doi.org/10.1155/2014/476068> (2014).
29. Papageorgiou, S. N., Xavier, G. M., Cobourne, M. T. & Eliades, T. Effect of orthodontic treatment on the subgingival microbiota: A systematic review and meta-analysis. *Orthod. Craniofac. Res.* 21, 175–185. <https://doi.org/10.1111/ocr.12237> (2018).
30. Pan, S. et al. Profiling of subgingival plaque biofilm microbiota in adolescents after completion of orthodontic therapy. *PLoS ONE* 12, e0171550. <https://doi.org/10.1371/journal.pone.0171550> (2017).
31. O'Reilly MM and Featherstone JD: Demineralization and remineralization around orthodontic appliances: An in vivo study. *Am J Orthod Dentofacial Orthop*
32. Topaloglu-Ak A, Ertugrul F, Eden E, Ates M and Bulut H: Effect of orthodontic appliances on oral microbiota-6 month follow-up. *J Clin Pediatr Dent* 35: 433-436, 2011.
33. Chang HS, Walsh LJ and Freer TJ: The effect of orthodontic treatment on salivary flow, pH, buffer capacity, and levels of mutans streptococci and lactobacilli. *Aust Orthod*
34. Kupietzky A, Majumdar AK, Shey Z, Binder R and Matheson PB: Colony forming unit levels of salivary Lactobacilli and Streptococcus mutans in orthodontic patients. *J Clin Pediatr Dent* 30: 51-53, 2005.
35. Jurela A, Repic D, Pejda S, Juric H, Vidakovic R, Matic I and Bosnjak A: The effect of two different bracket types on the salivary levels of S mutans and S sobrinus in the early phase of orthodontic treatment. *Angle Orthod*.
36. Effect of fixed orthodontic treatment on oral microbiota and salivary proteins DIAN JING¹, JIN HAO², YU SHEN¹, GE TANG¹, LEI LEI³ and ZHIHE ZHAO, *EXPERIMENTAL AND THERAPEUTIC MEDICINE* 17: 4237-4243, 2019
37. Pellegrini P, Sauerwein R, Finlayson T, McLeod J, Covell DA Jr, Maier T, Machida CA: Plaque retention by self-ligating vs elastic orthodontic brackets: Quantitative comparison of oral bacteria and detection with adenosine triphosphate-driven bioluminescence. *Am J Orthod Dentofacial Orthop* 135: 426-427. e1-e9, 2009
38. Akin M, Tezcan M, Ileri Z and Ayhan F: Incidence of white spot lesions among patients treated with self- and conventional ligation systems. *Clin Oral Invest*
39. Peros K, Mestrovic S, Anic-Milosevic S and Slaj M: Salivary microbial and nonmicrobial parameters in children with fixed orthodontic appliances. *Angle Orthod* 81: 901-906, 2011.
40. Lara-Carrillo E, Montiel-Bastida NM, Sánchez-Pérez L and Alanís-Tavira J: Effect of orthodontic treatment on saliva, plaque and the levels of Streptococcus mutans and Lactobacillus. *Med Oral Patol Oral Cir Bucal* 15: e924-e929, 2010
41. Lucchese, A., Bondemark, L., Marcolina, M. & Manuelli, M. Changes in oral microbiota due to orthodontic appliances: A systematic review. *J. Oral Microbiol.* 10, 1476645

42. Klaus, K.; Eichenauer, J.; Sprenger, R.; Ruf, S. Oral microbiota carriage in patients with multibracket appliance in relation to the quality of oral hygiene. *Head Face Med.* 2016, 12, 1–7.
43. Topaloglu-Ak, A.; Ertugrul, F.; Eden, E.; Ates, M.; Bulut, H. Effect of Orthodontic Appliances on Oral Microbiota—6 Month Follow-up. *J. Clin. Pediatr. Dent.* 2011, 35, 433–436
44. Andrucioi, M.C.D.; Nelson-Filho, P.; Matsumoto, M.A.N.; Saraiva, M.C.P.; Feres, M.; De Figueiredo, L.C.; Martins, L.P. Molecular detection of in-vivo microbial contamination of metallic orthodontic brackets by checkerboard DNA-DNA hybridization. *Am. J. Orthod. Dentofac. Orthop.* 2012, 141, 24–29
45. The Oral Microbiota Changes in Orthodontic Patients and Effects on Oral Health: An Overview Maria Contaldo 1,* , Alberta Lucchese 1 , Carlo Lajolo 2 , Cosimo Rupe 2 , Dario Di Stasio 1 , Antonio Romano 1 , Massimo Petrucci 3 and Rosario Serpico 1
46. Zharmagambetova A, Tuleutayeva S, Akhmetova S, Zharmagambetov A. microbiological aspects of the orthodontic treatment. *Georgian Med News*;264:39-43. 2017
47. Arendorf T, Addy M. Candidal carriage and plaque distribution before, during and after removable orthodontic appliance therapy. *J Clin Periodontol*;12(5):360-368. 1985.
48. Frequency of Pathogenic Microorganisms in Removable Orthodontic Appliances and Oral Mucosa in Children Marisela Rodríguez-Rentería / Raúl Márquez-Preciado/ Marine Ortiz-Magdaleno / Josué Bermeo-Escalona/ Luis Octavio Sánchez-Vargas, *The Journal of Clinical Pediatric Dentistry* Volume 45, Number 2/2021
49. Biofilm Associated Microorganisms on Removable Oral Orthodontic Appliances in Children in the Mixed Dentition Pathak A K/ Sharma D S, *The Journal of Clinical Pediatric Dentistry* Volume 37, Number 3/2013
50. Batoni G, Pardini M, Giannotti A, Ota F, Giuca MR, Gabriele M, Campa M, Senesi S. Effect of removable orthodontic appliances on oral colonisation by mutans streptococci in children; 109(6):388-92. 2001
51. Sumi Y, Kogami H, Ohtsuka Y, Kakinoki Y, Haruguchi Y, Miyamoto H. High correlation between the bacterial species in denture plaque and pharyngeal microflora. *Gerodontology*; 20:84-7. 2003
52. Ishikawa H, Aiba Y, Nakanishi M, Oh-Haashi Y, Koga Y. Suppression of periodontal pathogenic bacteria in the saliva of human by the administration of lactobacillus salivarius. *J Jap Soc Periodontol*; 45: 105-112. 2003
53. Effect of Removeable Orthodontic Appliance on Oral PH, Candida Albicans, Candida Dubliensis, and Streptococcus Mutans Count Farrokh Kolahi Aval1 , Negar Moghaddasi2 , Tara Azimi3 , Zahra Nematollahi4 , Abdolreza Jamilian5, *EAS J Dent Oral Med*; Vol-4, Iss-1 (Jan-Feb, 2022): 20-25
54. Hibino, K., Wong, R. W., Haegg, U., & Samaranayake, L. P. (2009). The effects of orthodontic appliances on Candida in the human mouth. *International journal of paediatric dentistry*, 19(5), 301-308
55. Muggiano, F., Quaranta, A., & Previati, M. (2014). Candida albicans: Colonization, role and effects of this opportunistic pathogen on orthodontic appliances.
56. Kundu, R., Tripathi, A. M., Jaiswal, J. N., Ghoshal, U., Palit, M., & Khanduja, S. (2016). Effect of fixed space maintainers and removable appliances on oral microflora in children: An in vivo study. *Journal*
57. In-vivo evaluation of the bacterial contamination and disinfection of acrylic baseplates of removable orthodontic appliances Fernanda Campos Rosetti Lessa,a Carla Enoki,a Izabel Yoko Ito,b Gisele Faria,c Mirian Aiko Nakane Matsumoto,d and Paulo Nelson-Filho. 2007 by the American Association of Orthodontists
58. Shi, B.; Wu, T.; McLean, J.; Edlund, A.; Young, Y.; He, X.; Lv, H.; Zhou, X.; Shi, W.; Li, H.; et al. The Denture-Associated Oral Microbiome in Health and Stomatitis. *mSphere* 2016
59. The Effect of Removable Orthodontic Appliances on Oral Microbiota: A Systematic Review Alessandra Lucchese 1,2,3,* , Chiara Bonini 4,5, Maddalena Noviello 4,5, Maria Teresa Lupo Stanghellini 6 , Raffaella Greco 6 , Jacopo Peccatori 4 , Antonella Biella 4 , Elena Tassi 4 , Valeria Beretta 4 , Fabio Ciceri 6 , Marta Marcolina 1,2,3, Enrico Gherlone 1,2,3 and Maurizio Manuelli, *Appl. Sci.* 2021, 11, 2881. <https://doi.org/10.3390/app11062881>.
60. Zhao R, Huang R, Long H, Li Y, Gao M, Lai W (2020) The dynamics of the oral microbiome and oral health among patients receiving clear aligner orthodontic treatment. *Oral Dis* 26:473–483
61. Changes in the Microbiome of the Inner Surface of Clear Aligners After Different Usage Periods Dong Yan1 · Yi Liu2 · Xiaoxia Che1 · Sicong Mi1 · Yao Jiao1 · Lijia Guo1 · Song Li1. *Current Microbiology* (2021) 78:566–575 <https://doi.org/10.1007/s00284-020-02308-5>
62. Yoshida A, Ansai T, Takehara T, Kuramitsu HK (2005) LuxSbased signaling affects Streptococcus mutans biofilm formation. *Appl Environ Microbiol* 71:2372–2380
63. Miethke, R. R., & Vogt, S. A comparison of the periodontal health of patients during treatment with the Invisalign® system and with fixed orthodontic appliances, *Journal of Orofacial Orthopedics*, 66(3), 2005
64. Zachrisson S, Zachrisson BU. Gingival condition associated with orthodontic treatment, *Angle Orthodontist*, 42(1), 1972, 26–3
65. An In Vivo Study on the Development of Bacterial Microbiome on Clear Orthodontic Retainer Kabilan Vellyagounder 1,* , Anil Ardeshta 2 and Serena Shah. *Dent. J.* 2022, 10, 239. <https://doi.org/10.3390/dj10120239>
66. Alterations of the oral microbiome in patients treated with the Invisalign system or with fixed appliances Qian Wang,a Jin-bao Ma,b Bo Wang,b Xue Zhang,c Yu-ling Yin,c and Hua Baia. 2019 by the American Association of Orthodontists
67. Forsberg CM, Brattstro'm V, Malmberg E, Nord CE. Ligature wires and elastomeric rings: two methods of ligation, and their association with microbial colonization of streptococcus mutans and lactobacilli. *Eur J Orthod* 1991;13:416-20
68. Peter Pellegrini,a Rebecca Sauerwein,b Tyler Finlayson,c Jennifer McLeod,d David A. Covell, Jr,e Tom Maier,f and Curtis A. Machidag, 2009 by the American Association of Orthodontists. doi:10.1016/j.ajodo.2008.08.018
69. Ireland, A.J.; Soro, V.; Sprague, S.V.; Harradine, N.W.T.; Day, C.; Al-Anezi, S.; Jenkinson, H.F.; Sherriff, M.; Dymock, D.; Sandy, J.R. The effects of different orthodontic appliances upon microbial communities. *Orthod. Craniofacial Res.* 2013, 17, 115–123.
70. Mártha, K.; L'orinczi, L.; Bică, C.; Gyergyay, R.; Petcu, B.; Lazăr, L.; Information, R. Assessment of Periodontopathogens in Subgingival Biofilm of Banded and Bonded Molars in Early Phase of Fixed Orthodontic Treatment. *Acta Microbiol. et Immunol. Hung.* 2016, 63, 103–113.

71. Türkkahraman, H.; Sayin, M.O.; Bozkurt, F.Y.; Yetkin, Z.; Kaya, S.; Onal, S. Archwire ligation techniques, microbial colonization, and periodontal status in orthodontically treated patients. *Angle Orthod.* 2005, 75, 231–236
72. De Souza, R.A.; Magnani, M.B.B.D.A.; Nouer, D.F.; Da Silva, C.O.; Klein, M.I.; Sallum, E.A.; Gonçalves, R.B. Periodontal and microbiologic evaluation of 2 methods of archwire ligation: Ligature wires and elastomeric rings. *Am. J. Orthod. Dentofac. Orthop.* 2008, 134, 506–512
73. Jing, D.; Hao, J.; Shen, Y.; Tang, G.; Lei, L.; Zhao, Z. Effect of fixed orthodontic treatment on oral microbiota and salivary proteins. *Exp. Ther. Med.* 2019, 17, 4237–4243.
74. Baka, Z.M.; Basciftci, F.A.; Arslan, U. Effects of 2 bracket and ligation types on plaque retention: A quantitative microbiologic analysis with real-time polymerase chain reaction. *Am. J. Orthod. Dentofac. Orthop.* 2013, 144, 260–267.
75. Uzuner, F.D.; Kaygisiz, E.; Çankaya, Z.T. Effect of the bracket types on microbial colonization and periodontal status. *Angle Orthod.* 2014, 84, 1062–1067.
76. Perera M, Al-Hebshi NN, Speicher DJ, Perera I, Johnson NW. Emerging role of bacteria in oral carcinogenesis: A review with special reference to perio-pathogenic bacteria. *J Oral Microbiol* 2016;8:32762.
77. Ahmet Kubilay Eroglu, Zeliha Muge Baka and Ugur Arslan, Comparative evaluation of salivary microbial levels and periodontal status of patients wearing fixed and removable orthodontic retainers ,*Am J Orthod Dentofacial Orthop* 2019;156:186-92
78. Bowen Li , Cailian Lu , XinhuiYa , XiaojunWu , GuilinWu & Xiantao Zeng, Effects of three orthodontic retainers on periodontal pathogens and periodontal parameters, *Scientific Reports* | (2023) 13:20709 | <https://doi.org/10.1038/s41598-023-46922-2>
79. Dhuha A. Al-Ali, D. Al Groosh, The influence of fixed orthodontic retainer on oral microbiota, *International Journal of Health Sciences*, 6(S2), 2214–2223. <https://doi.org/10.53730/ijhs.v6nS2.5483>.
80. Kabilan Velliyagounder , Anil Ardehna and Serena Shah, An In Vivo Study on the Development of Bacterial Microbiome on Clear Orthodontic Retainer, *Dent. J.* 2022, 10, 239
81. Sila Bilgin Yenera ; Om ur Polat Ozsoy, Quantitative analysis of biofilm formation on labial and lingual bracket surfaces ,*Angle Orthod.* 2020;90:100–108
82. Maria Francesca SFONDRINI , Maurizia DEBIAGGI² , Francesca ZARA , Roberto BRERRA , Mario COMELLI³ , Marco BIANCHI⁴ , Sara Ramella POLLONE⁵ , Andrea SCRIBANTE, Influence of lingual bracket position on microbial and periodontal parameters in vivo ,*J Appl Oral Science*, 2012;20(3):357-61.
83. Anadha N. Gujar, Anwar Al-Hazmi, A. Thirumal Raj, and Shankargouda Patil , Microbial profile in different orthodontic appliances by checkerboard DNA-DNA hybridization: An in-vivo study, *Am J Orthod Dentofacial Orthop* 2020;157:49-58
84. A. Demling, C. Demling, R. Schwestka-Polly , M. Stiesch and W. Heuer, Influence of lingual orthodontic therapy on microbial parameters and periodontal status in adults ,*European Journal of Orthodontics* 31 (2009) 638–642
