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The influence of fixed orthodontic retainer on oral microbiota

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***Abstract*---**Background: The oral cavity is a wide versatile microbial community in which many microbial species coexist in harmony. However, with certain circumstances, i.e. orthodontic appliances, pathogenic biofilm outgrowth occurs. The aim: The aim of this study was to compare opportunistic pathogens, associated with dental caries and periodontal disease in addition to Candida albicans, between healthy individuals and patient wearing fixed orthodontic retainers. Method: Fourty eight subjects were recruited andwere divided into retainer wearers and control cohort groups. Biofilm samples from the lingual surface of the mandibular anterior teeth of the control group and the fixed retainer surface were evaluated to detect Streptococcus mutans, Lactobacillus acidophilus, Aggrigatibacter actinomycetemcomitans, Fusobacterium nucleatum and C. albicans. Additionally, plaque (PI) and gingival (GI) indices were measured at the mandibular anterior teeth in both groups. The results: The results showed a significant increase in total micobial count in retainer group ($p<0.001$). The prevalence of microbial cultivity was higher in retainer group with a significant increase in PI and GI. Moreover, there was a high correlation between these genera and the GI. The conclusion: fixed retainer may increase cariogenic and periodontal pathogens and compromise oral health.. Further studies on improving the fixed retainer material and plaque control measures in retainer wearers are needed.

***Keywords*---**fixed retainer, opportunistic pathogens, periodontal pathogens, plaque gingival index.

Introduction

Retention is the step of orthodontic treatment that aims to preserve teeth in their corrected positions after active orthodontic treatment [1,2]. This is by preventing the teeth from rebound to their pretreatment positions by the action of periodontal, occlusal, soft tissue forces and continued dentofacial growth [3]. Retainers permit reorganization of the gingival and periodontal tissues, lessen the effect of changes due to growth, allow the adaptation of neuromuscular apparatus to the treated tooth position and maintain teeth with relatively high risk of relapse i.e. compromised plan for esthetic reasons [4]. There are basically two types of retainers, the removable and fixed orthodontic retainers. Occationally, the removable orthodontic appliances may be beneficial in retention as opposed to intra-arch instability and may serve as retainers in patients with growth issues i.e. modified functional appliances or part-time headgear [5]. However, if the case requires a permanent retention, a fixed retainer could be used which may be a probable approach for intra-arch retention when irregularity in a specified area is likely to be an issue [6]. In spite of the priority of the fixed retainer for long term retention, patient satisfaction and appliance invisiblility with less patient compliance, it has some drawbacks such as, bonding technique sensitive, time-consuming procedure i.e. passive positioning on the lingual surface, possibility of wire fracture or bond failure and the prospect of causing periodontal issues by compromising the oral hygiene [7]. Furthermore, the fixed retainer wire component can act as a shelter site for bacteria adhesion, biofilm and dental calculus formation that eventually leads to caries and periodontal diseases [8]. The oral ecosystem comprises of oral microflora, which harbors more than 700 different bacterial species in addition to fungi and viruses. Despite the fact that the bulk of these microbiota are approved as beneficial and commensally, part of them are in charge of oral infections which varying from tooth cavities to periodontal diseases and gum related lesions [9]. The major notorious bacteria for dental caries are *Streptococcus mutans*, and Lactobacilli [10,11,12].The *S. mutans*; that is a normal oral cavity inhabitant; is the most cariogenic type of all oral streptococci that can cause initiation and progression of dental caries[13]. Another important participant of the oral microflora is the *Aggrigatibacter actino-mycetemcomitans* which is considered as one of the initial colonisers for periodontal disease in juveniles (localized aggressive periodontitis LAP) and adolescent individuals [14]. Additionally, *Fusobacterium nucleatum*, is recoginised as a key player in biofilm figuration by its role in "bridging microorganism" by which enhances the adhesion and incorporation of pathogens responsible for periodontal lesions into the oral biofilm [15,16]. To the authors' knowledge, there is limited studies regarding the effect of fixed orthodontic retainer on the oral microbial ecosystem; therefore, the aim of this study was to compare the dental caries and periodontal pathogens i.e. *S. mutans*, *L. acidophillus*, *A. actino-mycetemcomitans* and *F. nucleatum*, in addition to *C. albicans* in fixed orthodontic retainer wearers and the oral caviy of a control group matching the same inclusion criteria.

Materials and method

Subjects

This cohort study involves 48 individuals that were divided into two groups, with an age range of 18- 37 years, who were recruited from private orthodontic clinic in Baghdad city/ Iraq. The first group consisted of 24 patients with fixed orthodontic retainer fitted on the lingual surfaces of the lower anterior teeth; whereas the other group consisted of 24 normal subjects matching the retainer group with regard to age, gender distribution and the inclusion criteria but without orthodontic appliances or retainers [17,18].

The criteria for participation were as followed: subjects fitted with multistrand 0.010*0.028 stainless steel fixed orthodontic retainers, (Braided Retainer Wire, Orthotechnology, Illinois, USA), for at least 3 months. Subject must be medically fit and healthy; had no signs of oral mucosal disease; had not received oral antimicrobials or antibiotic therapy for at least 3 months before data collection; not pregnant or lactating; and do not smoke [19,20]. The study was approved by the board ethical committee at the College of Dentistry/ University of Baghdad. (issue no.182, date 28/1/2020). An information sheet and written informed consents was obtained from the patients/ participants involved before patient recruiting process.

Methods

Sterile swabs sticks (Bhubaneswar, Odisha, India) were used to sample the lingual surface of the mandibular anterior teeth in the control group and the the fixed retainer surface that attached to the mandibular anterior teeth in retainer group. In the retainer group, a braided stainless steel wire (Orthotechnology) was bonded to the lingual surface of the mandibular six anterior teeth using flowable composite adhesive (3M Unitek, Monrovia, California). Each swab was immediately inserted in a 10ml sterile tube (sterile plain tube, Biozek, Netherland) contained 5 ml of brain heart infusion broth (BHI, Oxoid, Leicestershire, UK), and were taken immediately to the laboratory for processing[21]. The samples were vortex-mixed for 1 min to make a homogenous solution. Ten-fold serial dilution was carried out in phosphate buffered saline (PBS, Sigma Aldrich, Missouri,USA) before the samples were cultured onto blood agar (Oxoid, Basingstoke, UK) to obtain the total anaerobic bacterial counts; mitis salivarius agar (MS, Himedia, Mumbai, India), a selective medium for streptococci[22]; tryptic soy serum Bacitracin- Vancomycin agar (PDH, England), a selective medium for *A. actinomycetemcomitans* [23,24]; De Man-Rogosa-Sharpe broth (Oxoid), a selective medium for *L. acidophilus* [25]; crystal violet-Erythromycin agar (CVE, avongem, UK), a selective media for *F. nucleatum* [26] and Soubraud Dextrous agar (Neogen, Heywood, UK) for *C. albicans* detection [27].

The plates and tubes were stored anaerobically in CO_2 incubator for 24 hrs, except tryptic soy serum Bacitracin-Vancomycin agar plates were kept for 72 hrs [28]. To identify the microorganism, microbial growth by cultural characteristics [22], Gram stain and biochemical tests using catalase, oxidase and coagulase tests was carried out [29]. In addition to that, Plaque index and Gingival index were

recorded according to Löe and Silness Plaque Index (PI) and Gingival Index (GI) [30].

Statistical analysis

All data analysis was performed with the Statistical Package for Social Sciences (SPSS Inc., version 26.0) and the level of statistical significance in all analyses was set to 0.05. Shappiro-Wilk test was used to test the normality of distribution of data which revieled a non parametric distribution. A Mann Whitney-U test was used to find the difference in CFU, PI and GI between the studied groups; and Chi-square test was used to detect any difference related to gender and age. The Spearman's rank correlation analysis was used to establish the correlation among the prevalence of studied bacteria and GI and PI.

Results

Data from 48 subjects were collected and analyzed. The overall gender and age comparison showed a non significant difference between the two groups (Data not included). Figure 1 shows the investigated genera isolated from the retainer and control cohort. There was no significant difference between the groups; however, the total microbial count was significantly higher in fixed retainer group.

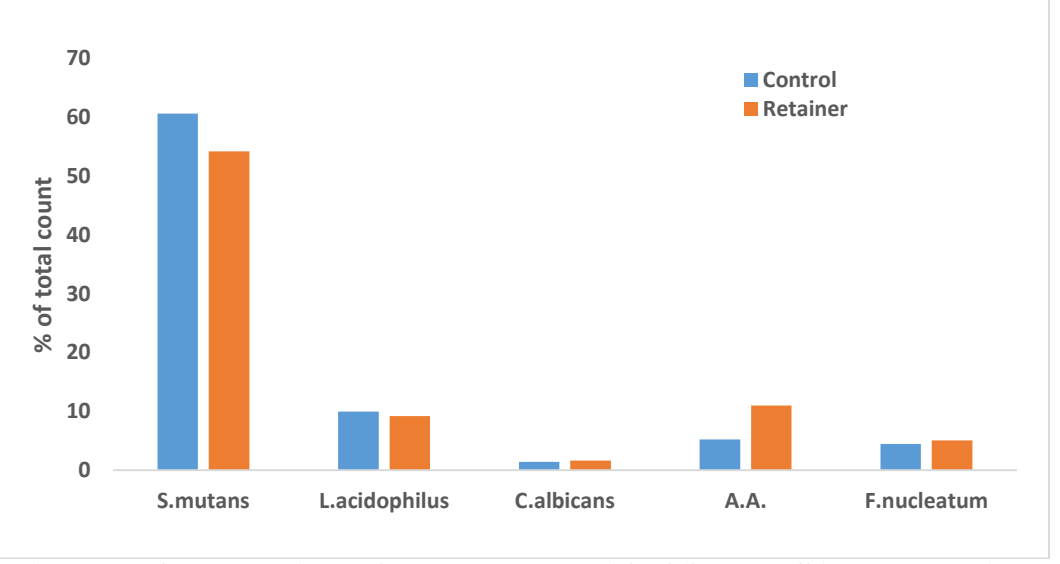

Figure 1: The proportions of *S. mutans*, *L. acidophilus*, *C. albicans*, *A. actinomycetemcomitans* and *F. nucleatum* in relation to total microbial count

The cultivity of these pathogens showed a higher proportions of all investigated genera in the fixed retainer group compared to controls. The prevalence of the isolated periodontal pathogens doubled in retainer group; additionally, the cultivation of *C. albicans* raised by approximately one third in retainer group (Figure 2).

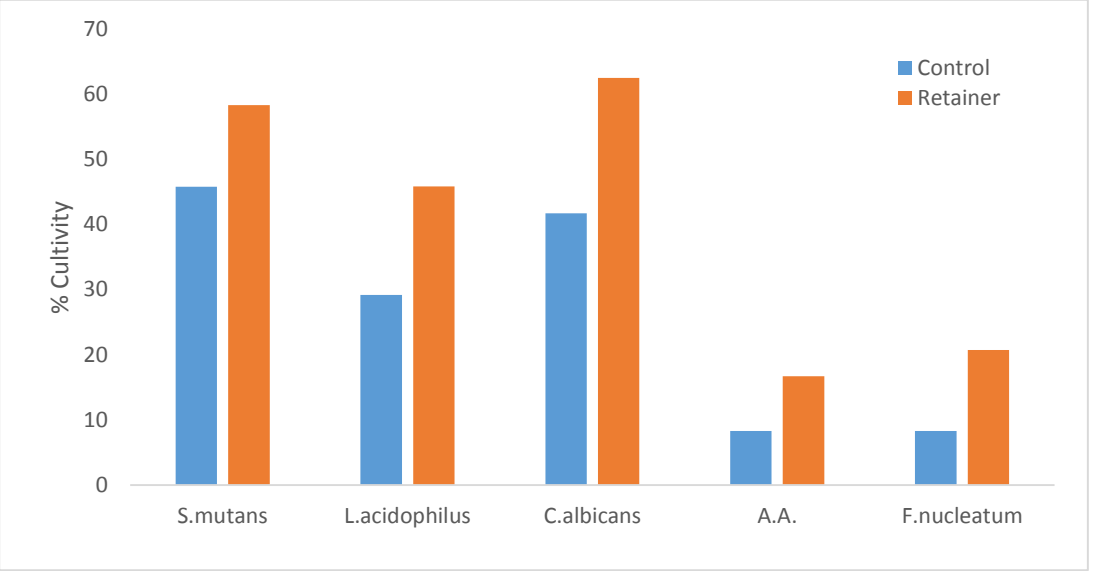

Figure 2: The prevalence of cultivated microorganisms harbouring the fixed retainer and the control groups (n=24).

Regarding plaque (PI) and gingival (GI) indices, there were statistically significant differences between the studied groups ($p<0.001$). Both indices were positively correlated to each other as seen in table 1.

Table 1: Correlation between PI and GI indices between retainer and control groups (n=24)

group		Min	Max	Mean ±SD	Mann-Whitney U	*P*-value	Spearman's correlation rho value	*P*-value
PI	C	0.111	1.111	0.440 ±0.257	22.5*	0.000	0.877*	0.000
	R	0.667	1.444	0.014 ±0.185				
GI	C	0.01	0.778	0.338 ±0.198	7.5*	0.000		
	R	0.556	1.333	0.965 ±0.192				

* Statistically significant difference ($p<0.001$)
C=control group, R=retainer group

The GI was positively associated with all investigated genera. Additionally, there was a positive correlation between the PI and the periodontal pthogens i.e. *F.nucleatum*, *A.actinomyces* and *L.acidophillus* (*p* values were 0.002 , 0.026 and 0.003 respectively), in addition to *C. albicans* (p=0.009) (Table 2).

Table 2: Correlation between PI and GI with the studied microorganisms (n=48)

	Genum	Spearman's correlation rho value	*p*-value		Genum	Spearman's correlation rho value	*p*-value
PI	Strept.	0.271	0.062	GI	Srept.	0.419*	0.003
	AA	0.419*	0.003		AA	0.293*	0.043
	Candida	0.374*	0.009		Candida	0.387*	0.007
	Lacto.	0.32*	0.026		Lacto.	0.405*	0.004
	Fuso.	0.439*	0.002		Fuso.	0.378*	0.008

*Stastictically significant ($p<0.05$)

Discussion

This cohort study designed to evaluate the effect of fixed orthdodntic retainers on selected organisms of the oral biofilm that considered as the main contributors in oral diseases such as dental caries, periodontitis, and oral candidiasis[31]. The sample number was 24 which yields the alpha to 0.05 and the power of the study to 0.8 assuming the effective threshold is 10000. Streptococci, lactobacilli, actinomyces, and fusobacteria were evaluated as these genera are often associated oral diseases like dental cavities and periodontal issues. Additionally candida was evaluated as it is the common opportunistic pathogen of the oral cavity and have been isolated from orthodontic retainers [32,33]. The data reveiled that there was an increase in all retrieved bacteria and *C. albicans*. It was found that the prevalence of this fungi increased in oral microbiota of orthodontic patients and may cause candida biofilm on orthodontic retainers. The results of the current study came in accordance with that reported by other studies [20,34,35,36].

The current results showed that the majority of microbial population was *S. mutans* and *L. acidophilus* in both groups. This came in agreement with previous authors who found that these pioneer bacteria compromised the majority of dental plaque and biofilm formed on the dental prosthesis [37,38,39]. This could be related to the surface irregularities that 'built-in' the fixed orthodontic retainers which furnish a good shelter for these aciduric and acidogenic bacteria [40] and Mummolo *et al.* who found that the levels of *S. mutans* and *Lactobacilli* in saliva increased in patients with fixed orthodontic appliance [18]. In addition to that, Türköz *et al.* found that the adherence of these bacteria increased after the application of thermoplastic orthodontic appliances [41].

However, the proportion of retrieved microorganisms in both groups was not significant. The data of the current study agreed with that of Jing *et al.* who reposted that the *S. mutans* population was not significantly changed during the first few months of orthodontic treatment with fixed appliance [42]. Moreover, the cultivity of the retrieved genera in the retainer group was higher. Several factors may attribute to that. It was proposed that the fixed or removable orthodontic appliances may impede the maintenance of oral hygiene by reducing the efficacy of normal innate plaque-removal mechanisms, such as salivary flow and the physiological cleansing movement of the tongue, resulting in plaque accumulation

and a potential damage to the periodontium [43]. This is specially true for *the A.actinomycetemcomitans* and *F. nucleatum*. it was claimed that these bacteria act as bridging species among the oral microbiome and heterotypic community formation during biofilm formation[44]. In addition to their abilities to aggregate with other bacteria, resulting in evolution of periodontitis [45]. This was clinically reflected in periodontal parameter in retainer group. Both plaque and gingival index were significantly higher and correlated with the studied genera. These outcomes came in agreement with Klingler And Jason Robert who found an increase of these bacteria in the saliva of patients with fixed orthodontic appliance [44]. Additionally, it was proposed that patients underwent orthodontic treatment were highly motivated regarding oral hygiene maintenance especially during the relatively short treatment recall visits, however, during retention, the follow-up visits are longer and the opportunity for microbial aggregation could be higher. This agreed with several auhtors who reported a positive correlation between plaque index and fixed retainer**s** [46,47].

Conclusion

The cultivity of the cariogenic and periodontal pathogens was higher in retainer group with a significant increase in the total microbial counts. That was reflected through the significant correlation of the studied genera with PI and GI, especially in retainer wearer group.

Conflict of Interest: Non
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References

1. Littlewood SJ, Kandasamy S, Huang G. Retention and relapse in clinical practice. Australian Dental Journal. 2017 Mar;62:51-7.
2. Kartal Y, Kaya B. Fixed orthodontic retainers: a review. Turkish journal of orthodontics. 2019 Jun;32(2):110.
3. Littlewood SJ, Millett DT, Doubleday B, Bearn DR, Worthington HV. Retention procedures for stabilising tooth position after treatment with orthodontic braces. Cochrane Database of Systematic Reviews. 2016(1).
4. Naidu S, Suresh A. A BRIEF OVERVIEW ON CLEFT LIP AND PALATE. Guident. 2018 Aug 1;11(9).
5. Proffit WR, Fields HW, Msd DM, Larson B, Sarver DM. Contemporary Orthodontics, 6e: South Asia Edition-E-Book. Elsevier India; 2019 Jun 29.
6. Graber LW, Vanarsdall RL, Vig KW, Huang GJ. Orthodontics-e-book: current principles and techniques. Elsevier Health Sciences; 2016 Jul 15.
7. Sobouti F, Rakhshan V, Saravi MG, Zamanian A, Shariati M. Two-year survival analysis of twisted wire fixed retainer versus spiral wire and fiber-reinforced composite retainers: a preliminary explorative single-blind randomized clinical trial. The korean journal of orthodontics. 2016 Mar 1;46(2):104-10.
8. Morita Y, Imai S, Hanyuda A, Matin K, Hanada N, Nakamura Y. Effect of silver ion coating of fixed orthodontic retainers on the growth of oral pathogenic bacteria. Dental materials journal. 2014 Mar 26;33(2):268-74.

9. Vasudevan R. Dental Plaques: Microbial community of the oral cavity. J Microbiol Exp. 2017;4(1):00100.
10. Laudenbach JM, Simon Z. Common dental and periodontal diseases: evaluation and management. Medical Clinics. 2014 Nov 1;98(6):1239-60.
11. Caufield PW, Schön CN, Saraithong P, Li Y, Argimón S. Oral lactobacilli and dental caries: a model for niche adaptation in humans. Journal of dental research. 2015 Sep;94(9_suppl):110S-8S.
12. Tanner AC, Kressirer CA, Rothmiller S, Johansson I, Chalmers NI. The caries microbiome: implications for reversing dysbiosis. Advances in dental research. 2018 Feb;29(1):78-85.
13. Al-mohammadawy ZH, Aljarah AK, Saad AM. Isolation and Identification of Streptococcus mutans from Dental Caries by Using Sm479 gene. Indian Journal of Public Health Research & Development. 2018 Oct 1;9(10).
14. Gholizadeh P, Pormohammad A, Eslami H, Shokouhi B, Fakhrzadeh V, Kafil HS. Oral pathogenesis of Aggregatibacter actinomycetemcomitans. Microbial pathogenesis. 2017 Dec 1;113:303-11.
15. Andersen RN, Ganeshkumar N, Kolenbrander PE. Helicobacter pylori adheres selectively to Fusobacterium spp. Oral microbiology and immunology. 1998 Feb;13(1):51-4.
16. Fujiwara N, Kitamura N, Yoshida K, Yamamoto T, Ozaki K, Kudo Y. Involvement of fusobacterium species in oral cancer progression: a literature review including other types of cancer. International Journal of Molecular Sciences. 2020 Jan;21(17):6207.
17. Alshehri EM, Al-Zahrani FA, Ibrahim WS. Association between Fixed Orthodontic Retainers and Gingival Health among Southern Region Population in Saudi Arabia-Cross sectional Study. Annals of Medical and Health Sciences Research. 2020.
18. Mummolo S, Nota A, Albani F, Marchetti E, Gatto R, Marzo G, Quinzi V, Tecco S. Salivary levels of Streptococcus mutans and Lactobacilli and other salivary indices in patients wearing clear aligners versus fixed orthodontic appliances: An observational study. PLoS One. 2020 Apr 24;15(4):e0228798.
19. Thornberg MJ, Riolo CS, Bayirli B, Riolo ML, Van Tubergen EA, Kulbersh R. Periodontal pathogen levels in adolescents before, during, and after fixed orthodontic appliance therapy. American journal of orthodontics and dentofacial orthopedics. 2009 Jan 1;135(1):95-8.
20. Al Groosh D, Roudsari GB, Moles DR, Ready D, Noar JH, Pratten J. The prevalence of opportunistic pathogens associated with intraoral implants. Letters in applied microbiology. 2011 May;52(5):501-5.
21. Abd FN, Luti K. An Exploitation of Interspecies Interaction for Promoting Bacteriocin Production by Local Isolate of Bacillus sp (Doctoral dissertation, M. Sc. Thesis. Department of Biotechnology, College of science, University of Baghdad).
22. Ismaïl R, Aviat F, Michel V, Le Bayon I, Gay-Perret P, Kutnik M, Fédérighi M. Methods for recovering microorganisms from solid surfaces used in the food industry: a review of the literature. International journal of environmental research and public health. 2013 Nov;10(11):6169-83.
23. AVILA-CAMPOS MJ, SIMIONATO MR, CAI S, MAYER MP, DE LORENZO JL, ZELANTE F. Virulence factors of Actinobacillus actinomycetemcomitans: other putative factors. Pesquisa Odontológica Brasileira. 2000;14:05-11.

24. Velliyagounder K, Ardeshna A, Koo J, Rhee M, Fine DH. The microflora diversity and profiles in dental plaque biofilms on brackets and tooth surfaces of orthodontic patients. Journal of Indian Orthodontic Society. 2019 Jul;53(3):183-8.
25. Zacharof MP, Lovitt RW. Bacteriocins produced by lactic acid bacteria a review article. Apcbee Procedia. 2012 Jan 1;2:50-6.
26. Shakhatreh MA, Khabour OF, Alzoubi KH, Masadeh MM, Hussein EI, Bshara GN. Alterations in oral microbial flora induced by waterpipe tobacco smoking. International journal of general medicine. 2018;11:47.
27. Gupta V, Abhisheik K, Balasundari S, Devendra NK, Shadab K, Anupama M. Identification of Candida albicans using different culture media and its association in leukoplakia and oral squamous cell carcinoma. Journal of oral and maxillofacial pathology: JOMFP. 2019 Jan;23(1):28.
28. Rurenga P, Raangs E, Singadji Z, Wekema-Mulder G, Veloo AC, Van Winkelhoff AJ. Evaluation of three selective media for isolation of A ggregatibacter actinomycetemcomitans. Journal of Periodontal Research. 2013 Oct;48(5):549-52.
29. Franco-Duarte R, Černáková L, Kadam S, S Kaushik K, Salehi B, Bevilacqua A, Corbo MR, Antolak H, Dybka-Stępień K, Leszczewicz M, Relison Tintino S. Advances in chemical and biological methods to identify microorganisms—from past to present. Microorganisms. 2019 May;7(5):130.
30. Löe H. The gingival index, the plaque index and the retention index systems. The Journal of Periodontology. 1967 Nov;38(6):610-6.
31. Salehi B, Kregiel D, Mahady G, Sharifi-Rad J, Martins N, Rodrigues CF. Management of Streptococcus mutans-Candida spp. oral biofilms' infections: Paving the way for effective clinical interventions. Journal of clinical medicine. 2020 Feb;9(2):517.
32. Marsh PD, Head DA, Devine DA. Dental plaque as a biofilm and a microbial community—Implications for treatment. Journal of oral biosciences. 2015 Nov 1;57(4):185-91.
33. Sun F, Ahmed A, Wang L, Dong M, Niu W. Comparison of oral microbiota in orthodontic patients and healthy individuals. Microbial pathogenesis. 2018 Oct 1;123:473-7.
34. Hibino K, Wong RW, Haegg U, Samaranayake LP. The effects of orthodontic appliances on Candida in the human mouth. International journal of paediatric dentistry. 2009 Sep;19(5):301-8.
35. Topaloglu-Ak A, Ertugrul F, Eden E, Ates M, Bulut H. Effect of orthodontic appliances on oral microbiota—6 month follow-up. Journal of Clinical Pediatric Dentistry. 2011 Jul 1;35(4):433-6.
36. Rammohan SN, Juvvadi SR, Gandikota CS, Challa P, Manne R, Mathur A. Adherence of Streptococcus mutans and Candida albicans to different bracket materials. Journal of pharmacy & bioallied sciences. 2012 Aug;4(Suppl 2):S212.
37. Pfeffer LA. Bacterial adherence of Streptococcus mutans and Lactobacillus acidophilus on poly-methyl methacrylate and thermoplastic polypropene used in orthodontic retention. InMasters Abstracts International 2011 (Vol. 50, No. 04).
38. Lee SH, Kim YJ. A comparative study of the effect of probiotics on cariogenic biofilm model for preventing dental caries. Archives of microbiology. 2014 Aug;196(8):601-9.

39. Lucchese A, Bondemark L, Marcolina M, Manuelli M. Changes in oral microbiota due to orthodontic appliances: a systematic review. Journal of oral microbiology. 2018 Jan 1;10(1):1476645.
40. Øilo M, Bakken V. Biofilm and dental biomaterials. Materials. 2015 Jun;8(6):2887-900.
41. Türköz Ç, Bavbek NC, Varlik SK, Akça G. Influence of thermoplastic retainers on Streptococcus mutans and Lactobacillus adhesion. American journal of orthodontics and dentofacial orthopedics. 2012 May 1;141(5):598-603.
42. Jing D, Hao J, Shen Y, Tang G, Lei L, Zhao Z. Effect of fixed orthodontic treatment on oral microbiota and salivary proteins. Experimental and therapeutic medicine. 2019 May 1;17(5):4237-43.
43. Shukla C, Maurya RK, Singh V, Tijare M. Evaluation of changes in Streptococcus mutans colonies in microflora of the Indian population with fixed orthodontics appliances. Dental research journal. 2016 Jul;13(4):309.
44. Klingler J. Prevalence of Aggregatibacter Actinomycetemcomitans and Fusobacterium Nucleatum Among Clinical Orthodontic Saliva Samples. 2019 (Doctoral dissertation, University of Nevada, Las Vegas).
45. Karched M, Bhardwaj RG, Asikainen SE. Coaggregation and biofilm growth of Granulicatella spp. with Fusobacterium nucleatum and Aggregatibacter actinomycetemcomitans. BMC microbiology. 2015 Dec;15(1):1-0.
46. Levin L, Samorodnitzky-Naveh GR, Machtei EE. The association of orthodontic treatment and fixed retainers with gingival health. Journal of periodontology. 2008 Nov;79(11):2087-92.
47. Di Venere D, Pettini F, Nardi GM, Laforgia A, Stefanachi G, Notaro V, Rapone B, Grassi FR, Corsalini M. Correlation between parodontal indexes and orthodontic retainers: prospective study in a group of 16 patients. Oral & Implantology. 2017 Jan;10(1):78.