Letters in Applied NanoBioScience
Platinum Open Access Journal (ISSN: 2284-6808)

Article
Volume 9, Issue 4, 2020, 1717 - 1724
https://doi.org/10.33263/LIANBS94.17171724

Proposal of a Model for Periodontal Prevention During Orthodontic Treatment

Cinzia Casu [1], Maria Giulia Nosotti [2], Andrea Baruffaldi [3], Luca Viganò [4]

[1] DDS, Private Dental Practice, Cagliari, Italy; e-mail: ginzia.85@hotmail.it (C.S.);
[2] RDH, Departement of Human Science, Innovation and Territory, Dental Hygiene School, University of Insubria, Varese, Italy; mg.nosotti@gmail.com (M.G.N.);
[3] DDS, Private Dental Practice, Piacenza, Italy; andrea@studiobaruffaldi.it (A.B.);
[4] DDS, Departement of Oral Radiology, San Paolo Dental Building, University of Milan, Milano, Italy; luca.vigano1@unimi.it (L.V.);
* Correspondence: Cinzia Casu ginzia.85@hotmail.it;

Scopus Author ID 57195993724
Received: 12.07.2020; Revised: 2.09.2020; Accepted: 5.09.2020; Published: 13.09.2020

Abstract: Background: Patients in orthodontic therapy often have an increase in gingival plaque and also in the periodontal bacterial load. The goal of the present work is to propose a model for the management of the periodontal tissues of these patients. Methods: 1) Patient 1 month after the insertion of the brackets is subjected to a test with molecular biology technique; 2) The patient is instructed to use a water jet daily, using it 2 minutes on both arches 2 times a day for a month; 3) The previously described microbiological test is performed again; 4) The reports obtained are compared and delivered to the patient after illustrating the problems; 5) if the percentage of periodontal pathogens exceeds 5% of the total, continue with phase 2 and 3, until the lowering of the same; 6) if lower, the test will be repeated after 6 months. Results: We reported 2 examples of the prevention model proposed in which patients improved significantly. Conclusions. The protocol we propose aims to limit periodontal damage possibly caused by orthodontic treatment. The proposed model could be the basis of clinical trials that also include a comparison with a control group.

Keywords: periodontal prevention; periodontal bacteria; orthodontic patients.

1. Introduction

Patients during fixed orthodontic therapy often could have an increase in gingival plaque production and also in the periodontal population [1,2,3]. A recent literature review has considered over 200 scientific articles on the subject and confirmed that, especially at the beginning of orthodontic therapy, we have an increase in the concentration of periodontal bacteria, which tends to decrease several months after therapy [4]. The most compromised element during orthodontic therapy is the central incisor. The authors said that the literature's studies analyzed overall 4 periodontal species: *Porphiromonas gingivalis*, *Prevotella intermedia*, *Aggregatibacter*, *Tanerella forsythensys* [4], but they have not found data on the globality of bacterial species.

Among the various home aids for the treatment and prevention of periodontal pathologies, we find the water jet [5,6,7]. The use of this device, according to a meta-analysis, is important to remove plaque, reduce the number of periodontal pathogens, inflammation mediators, and, for these reasons, gingivitis, and bleeding [8].

An even more recent review work (2018), which examined 22 randomized clinical trials, concluded that the most effective interdental cleaning methods are interdental brushes and hydropulsors, resulting in a reduction of the periodontal gingival indices of 64 respectively, 7% and 27.4% [9].

The use of the manual or electric toothbrush, interdental brushes, and superfluous are indispensable but not sufficient tools to perform good home oral hygiene [10], such as to allow the maintenance of bacterial plaque at acceptable levels in orthodontic patients [11]. The goal of this work is to propose a model for the management of the periodontal tissues of these patients.

2. Materials and Methods

The proposed model is divided into several phases:

1) Patient 1 month after the insertion of the brackets is subjected to a test with molecular biology technique to evaluate the presence of 12 species pathogenic periodontal bacteria and total bacterial load. The insertion of brackets could be worse the periodontal status [12].
2) The patient is instructed to use a water jet daily, using it 2 minutes on both arches 2 times a day for a month. As suggested by a recent review of the literature, it has been observed that the water jet is more effective than the bottle brush [9].
3) The previously described microbiological test is carried out.
4) The reports obtained are compared and delivered to the patient after illustrating the problems
5) If pathogenic bacterial species are present above a concentration of 100.000, or the percentage of periodontal pathogens exceeds 5% of the total, continue with phase 2 and 3, until the lowering of the same. A percentage very high of the periodontal pathogen, such as *P. gengivalis* is connected with the worsening of periodontal status [13].
6) If the previous conditions are not present, the test will be repeated every 6 months to verify the maintenance of periodontal microbial stability.

3. Results and Discussion

We want to provide two examples of applications of the model described.

Example 1. A 15-year-old female patient came to our observation for a follow-up visit. The girl in good general health is on fixed orthodontic therapy. After professional oral hygiene, we decided to make an assessment of the periodontal microbiological status, using a molecular biology method performed by Biomolecular Diagnostic laboratory, Firenze, Italy. The sampling for biomolecular analysis was carried out with a sterile paper cone, inserted in the gingival sulcus of element 11, and left inside for 20 seconds. Subsequently, it was placed inside a sterile test tube and closed in the special kit provided by the laboratory. We evaluated the total microbial load, the percentage of periodontal pathogens on the total of bacteria. We analyzed 15 pathogenic species. 8 of these 15 belong to the red complex (*Aggregatibacter, T. denticola, T. forsythensys, P. gengivalis, P. endodontalis, P. micros, F. alocis, Synergisteses*); 4 belong to the orange complex (*P. intermedia, F.nucleatum, C. rectus, L. hofstadii, E. corrodens, C. homonis*); 2 belong to the green complex (*E.corrodens, C.hominis*).

The results of this test showed that the total bacterial load was 1.31 10^7, while the percentage of pathogens was 15.23% (Fig 1,2). The bacteria most present were *E. corrodens* and *F.alocis*. It was decided to offer the patient the use of a home water jet (SoWash, Water powered, Salerno, Italy) in an attempt to reduce the bacterial load, at least 2 times a day for a

month of treatment, for a total of 4 minutes of daily application. Subsequently, another test was performed for molecular microbiological analysis, in the same way as above. The test revealed a total charge of 2.09 10^9, with a percentage of pathogens of 9.3% (fig 3,4). The percentage of pathogens indicates that treatment with a water jet must be continued, but there is an important improvement in bacterial load decrease.

Aggregatibacter actinomycetemcomitans	< 1.00 * 10 ^{2}			0.0000
Porphyromonas gingivalis	< 1.00 * 10 ^{2}			0.0000
Porphyromonas endodontalis	4.34 * 10 ^{3}		++	0.0331
Tannerella forsythensys	< 1.00 * 10 ^{2}			0.0000
Treponema denticola	< 1.00 * 10 ^{2}			0.0000
Peptostreptococcus micros	< 1.00 * 10 ^{2}			0.0000
Filifactor alocis	5.88 * 10 ^{4}		++	0.4489
Synergistetes	< 1.00 * 10 ^{2}			0.0000
Prevotella intermedia	< 1.00 * 10 ^{2}			0.0000
Fusobacterium nucleatum ssp	< 1.00 * 10 ^{2}			0.0000
Campylobacter rectus	2.69 * 10 ^{4}		++	0.2053
Rothia dentocariosa	5.37 * 10 ^{3}		++	0.0410
Leptotrichia hofstadii	1.34 * 10 ^{2}		+	0.0010
Eikenella corrodens	1.87 * 10 ^{6}		+++	14.2748
Cardiobacterium hominis	2.95 * 10 ^{4}		++	0.2252
Carica batterica totale	1.31 * 10 ^{7}			100.0000

Figure 1. Report of the biomolecular test, patient 1.

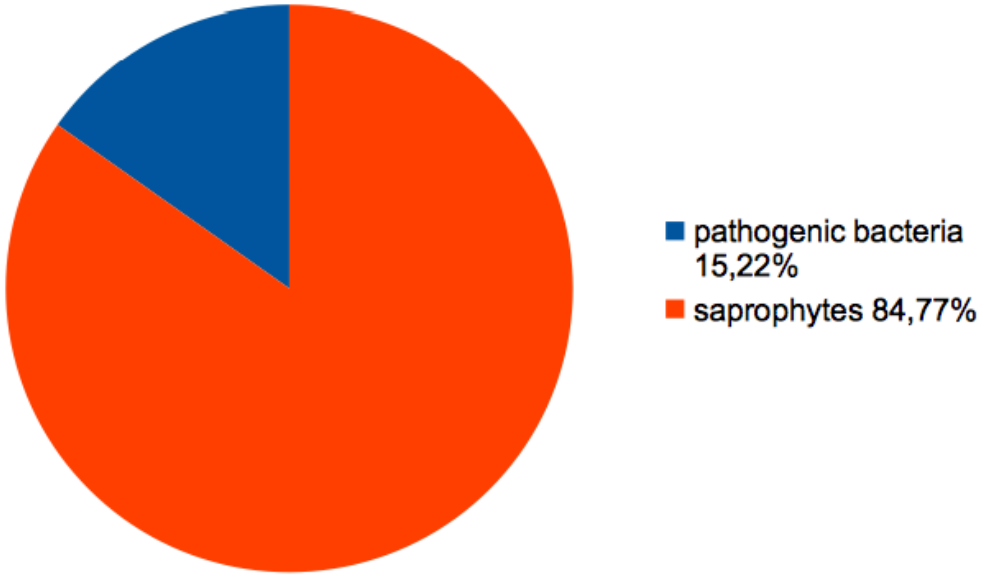

Figure 2. Percentage of pathogenic bacteria before water jet daily treatment, patient 1.

Aggregatibacter actinomycetemcomitans	< 1.00 * 10 ^{2}			0.0000
Porphyromonas gingivalis	< 1.00 * 10 ^{2}			0.0000
Porphyromonas endodontalis	7.30 * 10 ^{3}		++	0.3493
Tannerella forsythensys	< 1.00 * 10 ^{2}			0.0000
Treponema denticola	3.41 * 10 ^{2}		+	0.0163
Peptostreptococcus micros	< 1.00 * 10 ^{2}			0.0000
Filifactor alocis	1.47 * 10 ^{4}		++	0.7033
Synergistetes	< 1.00 * 10 ^{2}			0.0000
Prevotella intermedia	< 1.00 * 10 ^{2}			0.0000
Fusobacterium nucleatum ssp	< 1.00 * 10 ^{2}			0.0000
Campylobacter rectus	< 1.00 * 10 ^{2}			0.0000
Rothia dentocariosa	< 1.00 * 10 ^{2}			0.0000
Leptotrichia hofstadii	< 1.00 * 10 ^{2}			0.0000
Eikenella corrodens	1.69 * 10 ^{5}		+++	8.0861
Cardiobacterium hominis	3.27 * 10 ^{3}		++	0.1565
Carica batterica totale	2.09 * 10 ^{6}			100.0000

Figure 3. Report of biomolecular test, after 1 month with water jet treatment, patient 1.

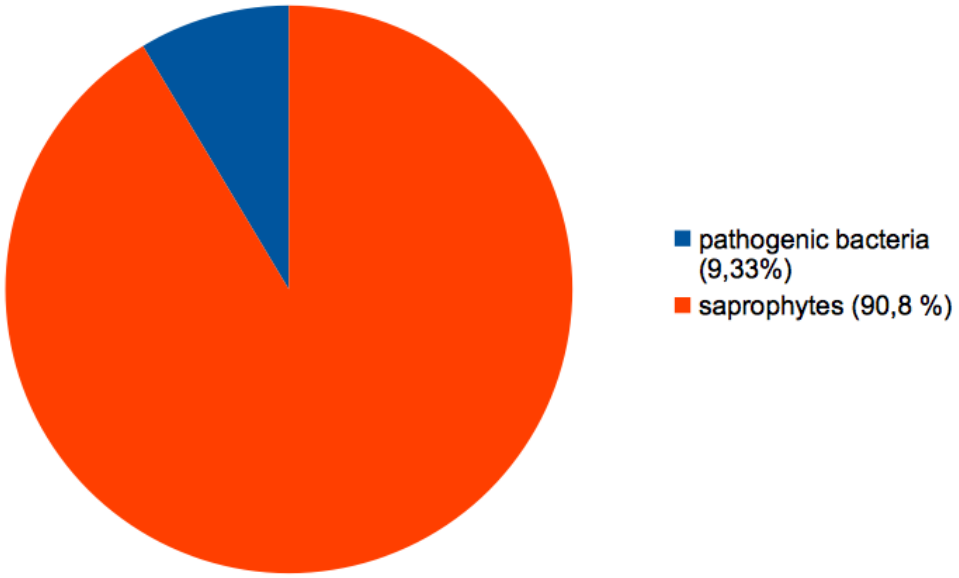

Figure 4. Percentage of pathogenic bacteria after 1 month with water jet, patient 1.

Example 2. A 12-year-old patient came to our observation, accompanied by his parents, for a check-up visit. The patient undergoes orthodontic treatment. Also, in this case, after professional oral hygiene, an oral microbiological evaluation with molecular biology was carried out. The withdrawal technique is identical to the previous case. The results revealed a percentage of 8.78% of periodontal pathogens (fig 5). No periodontal pathogen occurred in a particularly high charge. Also, in this case, the use of a water jet (SoWash, Water powered, Salerno, Italy) has been suggested twice a day for a month. No periodontal pathogen occurred in a particularly high charge. This time a non-electric water jet was proposed, which simply works with water pressure, connecting it directly to the tap. It does not require particular maintenance as it is free of electric circuits or batteries, eco-friendly, and allows you to adjust both the temperature and the pressure of the water jet, directly from the tap.

After one month, the test was performed again. The percentage of periodontal pathogens decreased significantly to 0.93% (fig 6). The percentage of pathogens indicates that treatment with a water jet can be stopped and re-evaluated after 6 months with a new molecular test.

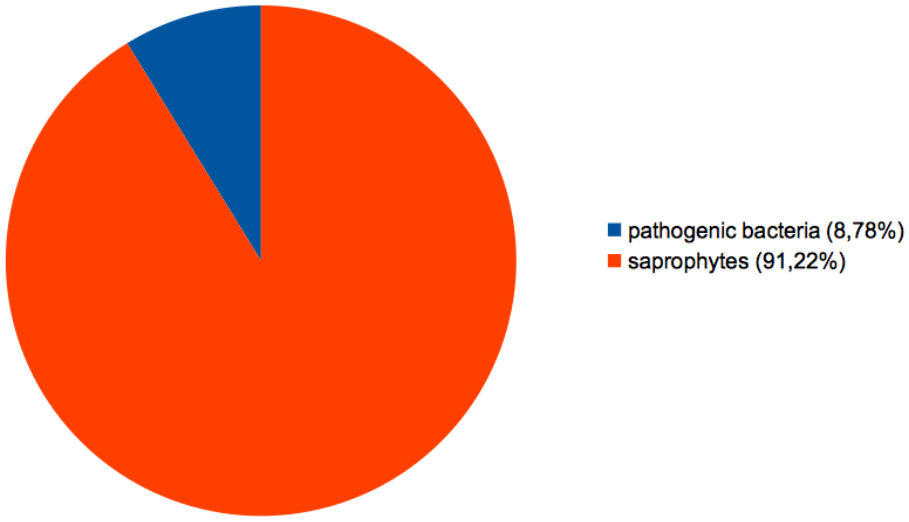

Figure 5. Percentage of pathogenic bacteria before water jet treatment, patient 2.

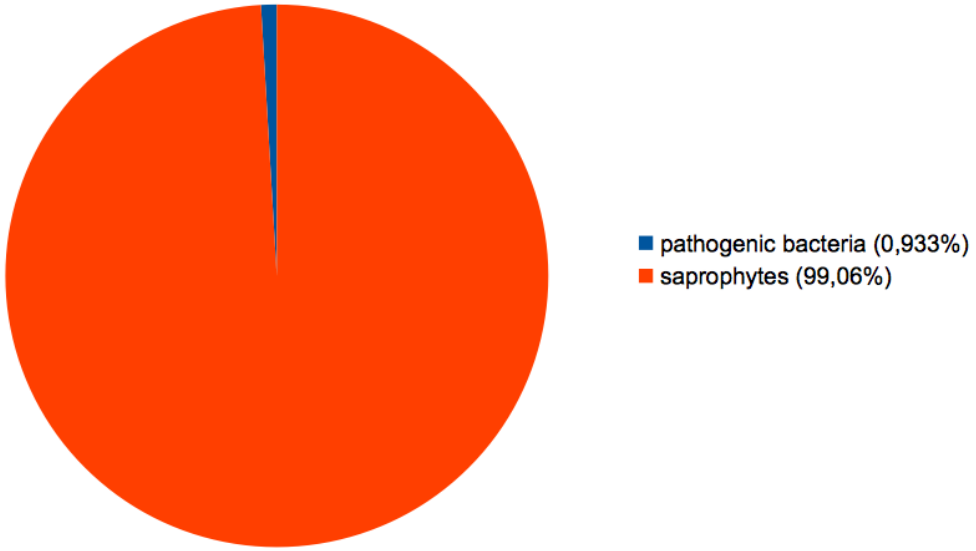

Figure 6. Percentage of pathogenic bacteria after treatment with water jet for a month, patient 2.

A work by Shirozaki *et al*. analyzed the levels of pro-inflammatory cytokine and periodontal indexes in patients with an orthodontic appliance. Bleeding values, plaque build-up, TNF alpha, IL 1-beta, have become worse on patients who have recently started therapy, and the authors no found statistically significant differences between fixed orthodontics and orthodontic realized by aligners [14]. The same results concluded Madriaga *et al*. [15]. Levels of some periodontal pathogens, especially red and orange complex, tend to increase in the first 60 days of orthodontic therapy [14,16].

The first point of our protocol involves the microbiological investigation of 12 bacterial species. In scientific literature, we have not found studies that have observed more than 6 bacterial species in the periodontal health of orthodontic patients [17,18,19].

In our opinion, the analysis of multiple bacterial species is important for a better evaluation of the microbiota of these patients. An assessment of the percentage of pathogenic species compared to all the bacteria found has never been documented in the literature for cases of patients on orthodontic therapy.

We know from the literature that a rate of pathogenic compared to the total of microorganisms greater than 5% is high and, therefore, a risk factor for the patient. In fact, in a study of over 1700 periodontal patients, it emerged that periodontal pathogens percentage found respect to total bacteria load were: *Aggregatibacter actinomycetemcomitans* 0.1%, *Campylobacter rectus* 2%, *Fusobacterium nucleatum* 8%, *Porphyromonas gingivalis* 6%, *Treponema denticola* 2% and *Tannerella forsythia* 1.5% [20].Periodontal pathogen concentrations above 10^5 have been correlated with the onset of periodontal problems [21,22].

Another of the points proposed by our protocol predicted the use of a manual and electric water jet for the maintenance of the periodontal state. Despite Mazzoleni *et al*. have not found very important results on home use of the water jet in orthodontic patients [23], previous literature reviews have found interesting data on the reduction of the bacterial population in patients who use water jet daily [8,9]. In the reported cases, a reduction in the general bacterial population and an extremely significant reduction in the percentage of pathogens was shown, which in the first case went from 15.23 to 9.3% and in the second case from 8.78% to 0,93%. We have not found in the literature any other data on the general lowering of the periodontal pathogenic load using the water jet. Some recent studies have

proposed other ways to reduce the bacterial load during orthodontic treatment, such as photobiomodulation, photodynamic therapy, and probiotics [24-28], but the use of a water jet is easier in our opinion overall for the patient. It also seems that the use of the eco-friendly non-electric device is even more performing for this purpose. In a view to reducing the consumption of energy resources and protecting the environment, that is also a new trend in biomedical science [29-31]. This fact could be interesting.

Another important point of our protocol is to demonstrate to the patient, through the evaluation of the reports with the patients themselves, how important their home contribution is for the maintenance of oral health during orthodontic therapy [32-34]. The use of bacterial tests can also be of great help in motivating the patient to oral hygiene itself [35].

4. Conclusions

Gingivitis and periodontitis arising during orthodontic treatment are not uncommon, as can be found in the literature [36,37,38,39]. Several principals have been used to limit this collateral damage [40,41,42]. But never a manual water jet. The protocol we propose aims to limit periodontal damage possibly caused by orthodontic treatment. The proposed model could be the basis of clinical trials that also include a comparison with a control group.

Funding

This research received no external funding.

Acknowledgments

This research has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Abbate, G.M.; Caria, M.P.; Montanari, P.; Mannu, C.; Orrù, G.; Caprioglio, A.; Levrini, L. Periodontal health in teenagers treated with removable aligners and fixed orthodontic appliances. *Journal of Orofacial Orthopedics* **2015**, *76*, 240-250, https://doi.org/10.1007/s00056-015-0285-5.
2. Zasčiurinskienė, E.; Basevičienė, N.; Lindsten, R.; Slotte, C.; Jansson, H.; Bjerklin, K. Orthodontic treatment simultaneous to or after periodontal cause-related treatment in periodontitis susceptible patients. Part I: Clinical outcome. A randomized clinical trial. *Journal of clinical periodontology* **2018**, *45*, 213-224, https://doi.org/10.1111/jcpe.12835.
3. Verrusio, C.; Iorio-Siciliano, V.; Blasi, A.; Leuci, S.; Adamo, D.; Nicolò, M. The effect of orthodontic treatment on periodontal tissue inflammation: A systematic review. *Quintessence International* **2018**, *49*, 69-77, https://doi.org/10.3290/j.qi.a39225.
4. 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* **2017**, *17*, https://doi.org/10.1186/s12903-017-0378-1.
5. Alharbi, M.; Farah, R.F. Effect of water-jet flossing on surface roughness and color stability of dental resin-based composites. *J Clin Exp Dent* **2020**, *12*, e169-e177, https://doi.org/10.4317/jced.56153.
6. Richards, D. The effectiveness of interproximal oral hygiene aids. *Evidence-Based Dentistry* **2018**, *19*, 107-108, https://doi.org/10.1038/sj.ebd.6401341.
7. Park, S.Y.; Kim, K.H.; Shin, S.Y.; Koo, K.T.; Lee, Y.M.; Chung, C.P.; Seol, Y.J. Decontamination methods using a dental water jet and dental floss for microthreaded implant fixtures in regenerative periimplantitis treatment. *Implant dentistry* **2015**, *24*, 307-316, https://doi.org/10.1097/ID.0000000000000208.
8. Jahn, C.A. The dental water jet: a historical review of the literature. *Journal of dental hygiene: JDH* **2010**, *84*, 114-120.

9. Kotsakis, G.A.; Lian, Q.; Ioannou, A.L.; Michalowicz, B.S.; John, M.T.; Chu, H. A network meta-analysis of interproximal oral hygiene methods in the reduction of clinical indices of inflammation. *Journal of Periodontology* **2018**, *89*, 558-570, https://doi.org/10.1002/JPER.17-0368.
10. Gallie A. Home use of interdental cleaning devices and toothbrushing and their role in disease prevention. *Evid Based Dent*. 2019;20(4):103-104. doi:10.1038/s41432-019-0069-7
11. Quaranta, A.; Marchisio, O.; D'Isidoro, O.; Genovesi, A.M.; Covani, U. Single-blind randomized clinical trial on the efficacy of an interdental cleaning device in orthodontic patients. *Minerva stomatologica* **2018**, *67*, 141-147, https://doi.org/10.23736/S0026-4970.18.04104-3.
12. Cardoso, M.D.A.; Saraiva, P.P.; Maltagliati, L.Á.; Rhoden, F.K.; Costa, C.C.A.; Normando, D.; Capelozza Filho, L. Alterations in plaque accumulation and gingival inflammation promoted by treatment with self-ligating and conventional orthodontic brackets %J Dental Press Journal of Orthodontics. **2015**, *20*, 35-41, https://doi.org/10.1590/2176-9451.20.2.035-041.oar.
13. Chigasaki, O.; Takeuchi, Y.; Aoki, A.; Sasaki, Y.; Mizutani, K.; Aoyama, N.; Ikeda, Y.; Gokyu, M.; Umeda, M.; Ishikawa, I.; Izumi, Y. A cross-sectional study on the periodontal status and prevalence of red complex periodontal pathogens in a Japanese population. *Journal of Oral Science* **2018**, *60*, 293-303, https://doi.org/10.2334/josnusd.17-0223.
14. Shirozaki, M.U.; da Silva, R.A.B.; Romano, F.L.; da Silva, L.A.B.; De Rossi, A.; Lucisano, M.P.; Messora, M.R.; Feres, M.; Novaes Júnior, A.B. Clinical, microbiological, and immunological evaluation of patients in corrective orthodontic treatment. *Progress in Orthodontics* **2020**, *21*, https://doi.org/10.1186/s40510-020-00307-7.
15. Pango Madariaga, A.C.; Bucci, R.; Rongo, R.; Simeon, V.; D'Antò, V.; Valletta, R. Impact of Fixed Orthodontic Appliance and Clear Aligners on the Periodontal Health: A Prospective Clinical Study. *Dentistry Journal* **2020**, *8*, https://doi.org/10.3390/dj8010004.
16. 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*. **2018**, *21*, 175-185, https://doi.org/10.1111/ocr.12237.
17. Sun, F.; Ahmed, A.; Wang, L.; Dong, M.; Niu, W. Comparison of oral microbiota in orthodontic patients and healthy individuals. *Microbial Pathogenesis* **2018**, *123*, 473-477, https://doi.org/10.1016/j.micpath.2018.08.011.
18. Kim, K.; Jung, W.-S.; Cho, S.; Ahn, S.-J. Changes in salivary periodontal pathogens after orthodontic treatment: An in vivo prospective study. *The Angle Orthodontist* **2015**, *86*, 998-1003, https://doi.org/10.2319/070615-450.1.
19. Joaquim CR, Miranda TS, Marins LM, *et al*. The combined and individual impact of diabetes and smoking on key subgingival periodontal pathogens in patients with chronic periodontitis. *J Periodontal Res*. 2018;53(3):315-323. doi:10.1111/jre.12516
20. Lauritano, D.; Martinelli, M.; Mucchi, D.; Palmieri, A.; Lo Muzio, L.; Carinci, F. Bacterial load of periodontal pathogens among italian patients with chronic periodontitis: a comparative study of three different areas. *Journal of biological regulators and homeostatic agents* **2016**, *30*, 149-154.
21. Vettore, M.V.; Leão, A.T.; Leal Mdo, C.; Feres, M.; de Figueiredo, L.C.; Sheiham, A. Periodontal bacterial load: a proposed new epidemiological method for periodontal disease assessment. *The journal of contemporary dental practice* **2010**, *11*, E049-056.
22. Collins, J.R.; Chinea, S.; Cuello, R.J.; Florian, A.P.; Palma, P.; Ambrosio, N.; Marín, M.J.; Figuero, E.; Herrera, D. Subgingival microbiological profile of periodontitis patients in Dominican Republic. *Acta odontologica latinoamericana : AOL* **2019**, *32*, 36-43.
23. Mazzoleni, S.; De Stefani, A.; Bordin, C.; Balasso, P.; Bruno, G.; Gracco, A. Dental water jet efficacy in the plaque control of orthodontic patients wearing fixed appliance: A randomized controlled trial. *J Clin Exp Dent* **2019**, *11*, e957-e963, https://doi.org/10.4317/jced.55411.
24. Al Nazeh, A.; Alshahrani, A.; Almoammar, S.; Kamran, M.A.; Togoo, R.A.; Alshahrani, I. Application of photodynamic therapy against periodontal bacteria in established gingivitis lesions in adolescent patients undergoing fixed orthodontic treatment. *Photodiagnosis and Photodynamic Therapy* **2020**, *31*, https://doi.org/10.1016/j.pdpdt.2020.101904.
25. Ren, C.; McGrath, C.; Gu, M.; Jin, L.; Zhang, C.; Sum, F.H.K.M.H.; Wong, K.W.F.; Chau, A.C.M.; Yang, Y. Low-level laser-aided orthodontic treatment of periodontally compromised patients: a randomised controlled trial. *Lasers in Medical Science* **2020**, *35*, 729-739, https://doi.org/10.1007/s10103-019-02923-0.
26. Rosa, E.P.; Murakami-Malaquias-Silva, F.; Schalch, T.O.; Teixeira, D.B.; Horliana, R.F.; Tortamano, A.; Tortamano, I.P.; Buscariolo, I.A.; Longo, P.L.; Negreiros, R.M.; Bussadori, S.K.; Motta, L.J.; Horliana, A.C.R.T. Efficacy of photodynamic therapy and periodontal treatment in patients with gingivitis and fixed orthodontic appliances: Protocol of randomized, controlled, double-blind study. *Medicine* **2020**, *99*, https://doi.org/10.1097/MD.0000000000019429.
27. Alqerban, A. Efficacy of antimicrobial photodynamic and photobiomodulation therapy against Treponema denticola, fusobacterium nucleatum and human beta defensin-2 levels in patients with gingivitis undergoing fixed orthodontic treatment: A clinic-laboratory study. *Photodiagnosis and Photodynamic Therapy* **2020**, *29*, https://doi.org/10.1016/j.pdpdt.2020.101659.

28. Pietri, F.K.; Rossouw, P.E.; Javed, F.; Michelogiannakis, D. Role of Probiotics in Oral Health Maintenance Among Patients Undergoing Fixed Orthodontic Therapy: a Systematic Review of Randomized Controlled Clinical Trials. *Probiotics and Antimicrobial Proteins* **2020**, https://doi.org/10.1007/s12602-020-09683-2.
29. Moro, G.; Bottari, F.; Van Loon, J.; Du Bois, E.; De Wael, K.; Moretto, L.M. Disposable electrodes from waste materials and renewable sources for (bio)electroanalytical applications. *Biosensors and Bioelectronics* **2019**, *146*, https://doi.org/10.1016/j.bios.2019.111758.
30. Montes-Cebrián, Y.; del Torno-de Román, L.; Álvarez-Carulla, A.; Colomer-Farrarons, J.; Minteer, S.D.; Sabaté, N.; Miribel-Català, P.L.; Esquivel, J.P. 'Plug-and-Power' Point-of-Care diagnostics: A novel approach for self-powered electronic reader-based portable analytical devices. *Biosensors and Bioelectronics* **2018**, *118*, 88-96, https://doi.org/10.1016/j.bios.2018.07.034.
31. Poderys, V.; Jarockyte, G.; Bagdonas, S.; Karabanovas, V.; Rotomskis, R. Protein-stabilized gold nanoclusters for PDT: ROS and singlet oxygen generation. *Journal of Photochemistry and Photobiology B: Biology* **2020**, *204*, https://doi.org/10.1016/j.jphotobiol.2020.111802.
32. Huang, J.; Yao, Y.; Jiang, J.; Li, C. Effects of motivational methods on oral hygiene of orthodontic patients: A systematic review and meta-analysis. *Medicine* **2018**, *97*, https://doi.org/10.1097/MD.0000000000013182.
33. Kumar, P.S.; Doshi, D.; Kulkarni, S.; Reddy, P.; Reddy, S.; Srilatha, A. Effect of motivation on oral hygiene and caries status among young adults in Hyderabad City. *Indian journal of dental research: official publication of Indian Society for Dental Research* **2019**, *30*, 15-20, https://doi.org/10.4103/ijdr.IJDR_220_17.
34. Marini, I.; Bortolotti, F.; Parenti, S.I.; Gatto, M.R.; Bonetti, G.A. Combined effects of repeated oral hygiene motivation and type of toothbrush on orthodontic patients: A blind randomized clinical trial. *The Angle Orthodontist* **2014**, *84*, 896-901, https://doi.org/10.2319/112113-856.1.
35. Montaldo, C.; Erriu, M.; Giovanna Pili, F.M.; Peluffo, C.; Nucaro, A.; Orrù, G.; Denotti, G. Microbial Changes in Subgingival Plaque and Polymicrobial Intracellular Flora in Buccal Cells after Fixed Orthodontic Appliance Therapy: A Preliminary Study. *International Journal of Dentistry* **2013**, *2013* https://doi.org/10.1155/2013/679312.
36. Zhao, L.; Wang, X.Y.; Xu, Y.; Meng, S. Relationship of orthodontic treatment and periodontal soft tissue health. *Hua Xi Kou Qiang Yi Xue Za Zhi* **2018**, *36,* 595-601, https://doi.org/10.7518/hxkq.2018.06.003.
37. Kossack, C.; Jost-Brinkmann, P.-G. Plaque and Gingivitis Reduction in Patients Undergoing Orthodontic Treatment with Fixed Appliances—Comparison of Toothbrushes and Interdental Cleaning Aids. *Journal of Orofacial Orthopedics / Fortschritteder Kieferorthopädie* **2005**, *66*, 20-38, https://doi.org/10.1007/s00056-005-0344-4.
38. Al-Moghrabi, D.; Pandis, N.; Fleming, P.S. The effects of fixed and removable orthodontic retainers: a systematic review. *Progress in Orthodontics* **2016**, *17*, https://doi.org/10.1186/s40510-016-0137-x.
39. Alhaija, E.S.A.; Al-Saif, E.M.; Taani, D.Q. Periodontal health knowledge and awareness among subjects with fixed orthodontic appliance *J Dental Press Journal of Orthodontics* **2018**, *23*, 40.e41-40.e49, https://doi.org/10.1590/2177-6709.23.5.40.e1-9.onl.
40. Erbe, C.; Klukowska, M.; Timm, H.C.; Barker, M.L.; van der Wielen, J.; Wehrbein, H. A randomized controlled trial of a power brush/irrigator/mouthrinse routine on plaque and gingivitis reduction in orthodontic patients. *The Angle Orthodontist* **2018**, *89*, 378-384, Https://doi.org/10.2319/022618-159.1.
41. Silvestrini Biavati, A.; Gastaldo, L.; Dessì, M.; Silvestrini Biavati, F.; Migliorati, M. Manual orthodontic vs. oscillating-rotating electric toothbrush in orthodontic patients: a randomised clinical trial. *European journal of paediatric dentistry* **2010**, *11*, 200-202.
42. Alp, S.; Baka, Z.M. Effects of probiotics on salivary Streptecoccus mutans and Lactobacillus levels in orthodontic patients. *Am J Orthod Dentofacial Orthop*. **2018**, *154*, 517-523, https://doi.org/10.1016/j.ajodo.2018.01.010.