Changes in oral microbiota due to orthodontic appliances: a systematic review

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**ABSTRACT**

**Background:** Oral microbiota has been at the center of cultural attention in recent years. In daily clinical practice, orthodontic appliances may be associated with an increased cariogenic risk and a worsening of preexisting periodontal diseases.

**Objective:** The purpose of this review is to investigate the available evidence regarding the association between orthodontic appliances and changes in the quality and quantity of the oral microbiota.

**Design:** The research included every article published up to October 2017 featuring the keywords ‘Orthodontic appliance* AND (microbiological colonization OR periodontal pathogen* OR Streptococcus mutans OR Lactobacillus spp. OR Candida OR Tannerella forsythia OR Treponema denticola OR Fusobacterium nucleatum OR Aggregatibacter actinomycetemcomitans OR Prevotella intermedia OR Prevotella nigrescens OR Porphyromonas gingivalis)’ and was conducted in the major medical databases. The methodological quality of selected papers was scored using the ‘Swedish Council on Technology Assessment in Health Care Criteria for Grading Assessed Studies’ (SBU) method.

**Results:** Orthodontic appliances influence the oral microbiota with an increase in the counts of *S. mutans* and *Lactobacillus* spp. and in the percentage of potentially pathogenic gram-negative bacteria.

**Conclusions:** There is moderate/high evidence regarding the association between orthodontic appliances and changes in the oral microbiota.

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**Introduction**

Periodontal health is crucial and requires special attention when performing an orthodontic treatment plan, both in adult and pediatric patients [1]. Preserving the integrity of periodontal tissues is one of the main concerns of orthodontics specialists, which has led to the definition of specific hygiene protocols for orthodontic patients [2]. Since 1985, the scientific community has been very concerned about the interaction between orthodontic devices and oral bacteria [3,4]; in fact, the first studies to analyze the oral microbiota and conventional braces (CB) took place in this period. In 2012, Freitas et al. published a systematic review regarding the alteration of the oral microbiota caused by fixed appliances [5]. The authors concluded that ‘The literature revealed moderate evidence that the presence of fixed appliances influences the quantity and quality of oral microbiota’. However, the authors included papers that analyzed bacteria from appliance surfaces and from oral mucosa, without distinction.

Furthermore, a significant number of studies have been published since 2012. Our review aims to update the research of Freitas et al., focusing on studies that have analyzed the microbiota collected from oral sites and not directly from appliances, and including all appliance types (self-ligating braces, invisalign aligners, sports-mouthguards, and other removable appliances) and not only fixed appliances.

Thus, the clinical research questions were as follows:

- Do orthodontic appliances influence the quality and quantity of the oral microbiota?
- What are the effects of orthodontic devices on the different bacterial species in the oral cavity?

**Materials and methods**

A search of the keywords Orthodontic appliance* AND (microbiological colonization OR periodontal pathogen* OR Streptococcus mutans OR Lactobacillus spp. OR Candida OR Tannerella forsythia OR Treponema denticola OR Fusobacterium nucleatum OR Aggregatibacter actinomycetemcomitans OR...
Prevalent intermedia OR Prevotella nigrescens OR Porphyromonas gingivalis was conducted in PubMed, PMC, Scopus, Lilacs, Scielo, Cochrane Trial Library, Web of Science. All articles published up to October 2017 were included. The Preferred Reporting Items for Reporting Systematic reviews and Meta Analyses protocol were adopted for this systematic review [6].

During the first phase, all the articles were selected by title and abstract by two of the authors and duplicate exclusion was performed. In the next phase, the full texts of potentially relevant papers were evaluated to determine if they met the eligibility criteria. Articles were selected on the basis of the criteria listed in Table 1. The article selection process is illustrated in Figure 1. Discussions were held to resolve any disagreements; when a resolution could not be found, a third review was consulted. Data extraction from the selected papers was performed independently by two review authors who adopted a template similar to that of Freitas et al. [5]. The template was adapted to the necessities of our study and is shown in Table 2 [5].

Extracted data included first author, year of publication, study design, sample size, age of the patients, type of appliance analyzed, collection time of the study, collection methods, microbial analysis methods, and quality of the study.

**Quality analysis**

The methodological quality is ‘the extent to which the design and conduct of a study are likely to have prevented systematic errors (bias)’. Variation in quality can explain variation in the results of studies included in a systematic review. More rigorously designed (better ‘quality’) trials are more likely to yield results that are closer to the ‘truth’ [7].

The methodological quality of selected papers was scored using the ‘Swedish Council on Technology Assessment in Health Care Criteria for Grading Assessed Studies’ (SBU) method, which was also used to assess the level of evidence for the conclusions of this review. The SBU method divided the methodological quality of the articles into three grades: grade A – high value of evidence, grade B – moderate value of evidence, and grade C – low value of evidence; once a score had been assigned to each study, the review’s level of evidence was stated in four grades: grade 1 – strong scientific evidence (at least two studies assessed at level A), grade 2 – moderate scientific evidence (one level A study and at least two studies at level B), grade 3 – limited scientific evidence (at least two studies at level B), and grade 4 – insufficient scientific evidence (fewer than two studies at level B) (Table 3–4) [8].

**Results**

From the initial 588 articles, 51 were selected [3,4,9–57].

**Quality of evidence**

In 37 of the 52 articles presented with moderate methodological quality [9–21,24–26,28,29,31–33,35–39,41–46,51–53,56,57], the major concern was the absence of repeatability tests. One article had a high quality [40] and the remaining 13 papers were classified as having a low quality [3,4,22,23,27,30,34,47–50,54,55]. Due to the lack of homogeneity in the study settings, a meta-analysis could not be applied and a systematic review realized.
| Reference | Study design | Sample size | Groups | Age | Appliance | T0 | T1 | T2 | T3 | T4 | Collection methods | Microbial analysis methods | Quality of the study |
|-----------|--------------|-------------|--------|-----|-----------|----|----|----|----|----|---------------------|--------------------------|----------------------|
| Al-Anezi [9] | RCT (cross-arch) | 24 | 1 | Mean: 12.6 years ± 1.01 month | SL braces + elastomeric modules | Before bonding | 3 months | Sterile paper points from the lateral incisors ligated with and without elastomeric modules | PCR + DGGE | B |
| Alves et al. [10] | RCT (split mouth) | 14 (6 M/8 F) | 1 | Mean: 17 years ± 2.6 months | CB/Steel ligatures vs. CB/elastomeric rings | Before bonding | 6 months | Sterilized periodontal curet 2 mm supragingival and 2 mm subgingival | PCR | B |
| Arab et al. [12] | Prospective study | 30 (6 M/24 F) | 1 | 12–18 years | CB | Before bonding | 6 weeks | 12 weeks | 18 weeks | Saliva collected by spitting into a sterile test tube for 10 min | Number CFU/ml was quantified | B |
| Arkan et al. [11] | RCT | 38 (20 M/18 F) | 2 | 4–10 years | Fixed and removable space maintainers | Before appliance of maintainers | 1 month | 3 months | 6 months | Saliva from fixed and removable space maintainers | Candida colonies were counted separately for each site by visual examination and expressed as CFU/mm² | B |
| Arslan et al. [13] | Prospective study | 42 (23 F/19 M) | 1 | Mean: 19.8 years | CB | 1 month before bonding | 1 month | 6 months | 12 months | DNA extracted from saliva, enamel surfaces of U5 and L5, and U1, and L1 adjacent to the braces with sterile wooden toothpicks (at T0 samples only from saliva and not from the teeth) | Candida identified by gram-staining, a germ-tube test, chlamydospore, and an API 20C AUX system (Bio-Mérieux, Marcy l’Etoile, France). | B |
| Baka et al. [14] | RCT (split mouth) | 20 (20 M) | 2 | Mean: 14.2 years ± 1.5 months | SL braces vs CB/steel ligature | Before bonding | 1 week | 3 months | Sterilized curettes from the labial surfaces of U2 and L2 left and right | DNA extracted from supragingival plaque samples (Dental blood and tissue kit) + real-time PCR | B |
| Reference          | Study design | Sample size (male/female) | Groups | Age               | Appliance                  | Collection time | Collection methods                                                                 | Microbial analysis methods | Quality of the study |
|--------------------|--------------|---------------------------|--------|-------------------|----------------------------|------------------|-------------------------------------------------------------------------------------|---------------------------|---------------------|
| Demling et al. [15] | Prospective  | 10 (8 F/2 M)              | 1      | Mean 29.0 years ± 4.7 months | Lingual braces             | Before bonding   | 3 months                                                                            | Samples of gingival crevicular fluid taken using sterile paper points. Buccal and lingual sites of U6 and L6, U4 and L4, U1 and L1. In extraction cases, the US and LS instead of the U4 and L4. | PCR                        | B                   |
| Demling et al. [16] | Prospective  | 20 (6 M/14 F)             | 1      | Mean 22.3 years ± 8.6 months | Lingual braces             | Before bonding   | 4 weeks                                                                            | Gingival crevicular fluid taken with sterile paper points at labial and lingual sites of U6 and L6, U4 and L4, and U1 and L1. In extraction cases, US and LS instead of U4 and L4. | DNA extracted with a QIAmp DNA Mini Kit + PCR | B                   |
| O’Ecole et al. [17] | Prospective  | 40 (27 M/33 F)            | 1      | Mean 9.9 years ± 1.2 months | Sport mouthguards          | Before mouthguard | 6 months 1 year 6 months without | Stimulate saliva with paraffin wax to chew and saliva collected for 5 min in a measuring cup | CFUs of SM counts per milliliter of saliva (E. coli) | B                   |
| Farhadian et al. [18] | RCT         | 66                         | 2      | Age ≤25 years       | Conventional removable retainers vs. removable retainers containing silver nanoparticles | 1 week after debonding 7 weeks after retainer delivery | Swab samples were taken from the maxillary palatal side | Number of SM CFU was counted with a digital colony counter | CFU count of SM and LB | B                   |
| Fonsberg et al. [19] | RCT (split mouth) | 12 (6 M/6 F)             | 1      | 12–14 years        | Ligature wires vs. elastomeric rings (CB) | 1 week before bonding  | Before bonding 4 weeks 10 weeks | Stimulated saliva samples collected with charcoaled points from U2 | CFU count of SM and LB | B                   |

(Continued)
| Reference          | Study design       | Sample size (male/female) | Groups                                                                 | Age                  | Appliance                                                                                     | Collection time                                                                 | Collection methods                                                                                   | Microbial analysis methods                                                                 | Quality of the study |
|--------------------|--------------------|---------------------------|------------------------------------------------------------------------|----------------------|-----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|----------------------|
| Ghijselings et al. [21] | Prospective study | 24 (10 M/14 F)            | G1: 10 (4 M/6 F) braces only; G2: 14 additionally treated with a headgear | Mean: 14.6 years ± 1.1 months | CB vs. CB + headgear                                                                         | Braces removal 3 months follow-up; 2 years follow-up                                        | Supragingival plaque removed by means of sterile curettes. Subgingival plaque examined by sterile paper points inserted per site (three mesially and three distally) and kept in place for 10 s. | Total number of, respectively, anaerobic and aerobic CFU was counted. Specific black-pigmented colonies on a nonselective anaerobic plate were counted | B                    |
| Hägg et al. [22]    | Prospective study | 27 (13 M/14 F)            | 1                                                                 | Mean: 15.5 years ± 2.3 months | CB                                                                                           | Examined 3 times during a 3-month follow-up; Examined 3 times during a 3-month follow-up; Examined 3 times during a 3-month follow-up | Imprint culture: sterile plastic foam pads dipped in Sabouraud’s broth and placed on the dorsum of the tongue. Oral rinse. Pooled plaque | Candida visual counting CFU: Yeast, gram-stain, germ tube test, and the API 20C AUX assimilation test | C                    |
| Hernández-Solis et al [23] | Prospective study | 60                         | 1                                                                 | 4–10 years                                      | Orthodontic appliance                                                                    | Before appliance 6 months                                                                       | Samples taken with a sterile swab rubbed over oral mucosa and the back of the tongue. | PCR                                                                   |                                                                  | C                    |
| Ireland et al. [24] | RCT (split mouth) | 24                         | 1                                                                 | 11–14 years                                     | SL braces + bands + bonded molar tubes to contralateral quadrants of the mouth + elastomeric ligature on one U2 bracket | Pre-bond-up at the molar separator appointment 3 months; Just prior of debonding 3 months post-debond 1 y post-debond | Supragingival plaque samples on molars (bands and bands) using sterile curettes and subgingivally using sterile paper points U2 (with or without elastomeric ligature): supragingival plaque collected adjacent to the bracket margins | PCR + microarray hybridization | B                    |
| Junfka et al. [25]  | Prospective study | 32                         | G1: 16 CB; G2: 16 esthetic braces                                      | 13–30 years                                      | CB vs. esthetic plastic braces                                                              | Before bonding 12 weeks                                                                         | Supragingival plaque samples on molars using sterile curettes and subgingivally using sterile paper points U2 (with or without elastomeric ligature): supragingival plaque collected adjacent to the bracket margins | PCR + cultivation method                                                                  | B                    |
| Reference               | Study design | Sample size (male/female) | Groups | Age | Appliance | T0 | T1   | T2 | T3 | T4 | Collection methods | Microbial analysis methods | Quality of the study |
|------------------------|-------------|--------------------------|--------|-----|-----------|----|------|----|----|----|---------------------|--------------------------|-----------------|
| Kim et al. [26]        | Prospective | 30                       | 1      | Mean: 16.7 years ± 6.5 months | CB  | Before bonding | 1 week | 3 months | 6 months | Sterile paper points from the distobuccal gingival crevice of the left U1, the left L1, the mesiobuccal gingival crevice of the left U6, and the left L6 | PCR | B |
| Kupietzky et al. [27]  | Prospective study (case control) | 64                       | 2      | G1: 32 braces G2: 32 control | CB  | G1: Before bonding | G2: 2 months before G1 | 2 months | Salivary collection and bacterial culture followed manufacturer’s instructions | LB and SM CFU were compared with standard densities | C |
| Lara-Carrillo et al. [28] | Prospective study | 30 (11 M/19 F)  | 1      | M mean: 16.5 years ± 3.7 months F mean: 16.5 years ± 5.5 months | CB  | Before bonding | 1 month | Canine retraction (placement of elastic chain in mouth) | Anterior segment retraction (placement of closing loops in mouth) | Buccal surface of U6, collected with a Q-6p | Dentocult® SM + Dentocult® LB | B |
| Lara-Carrillo et al. [29] | Prospective study | 34 (14 M/20 F)  | 1      | Mean: 16.7 years ± 5.2 months | CB  | Before bonding | 1 month | | | Unstimulated saliva from inner mucosa Stimulated saliva by chewing Sterilized cotton swab on U6 | SM Dentocult® SM LB: Dentocult® LB | B |
| Leung et al. [30]      | Prospective study | 27 (14 M/13 F)  | 1      | Mean: 14.9 years | CB  | Before bonding | At least 4 weeks after (mean 7 weeks) | | | BCC: sterile cytologic brushes on both cheeks. Plaque samples were obtained on the buccal surfaces of the 4s premolars. Supragingival and subgingival plaque removed with a sterile periodontal curette | PCR + FISH | C |
| Levini et al. [31]     | RCT         | 77 (32 F/25 M)           | 3      | Mean: 24.3 years | Invalign | Begin of the treatment | 1 month | 3 months | | Sterile paper points into the deepest part of the gingival sulcus for 30 s. Sites: U6 right (Site 0) and U1 left (Site 1) | Real-time PCR | B |
| Liu et al. [32]        | Prospective study | 17                       | 1      | Mean: 12.6 years | CB  | Before bonding | 1 month | 3 months | 6 months | Sterile probe passed along the supragingival smooth surface of the upper right teeth | Levels of total viable count, total Streptococci and SM in dental plaque + AP-PCR | B |

(Continued)
| Reference | Study design | Sample size (male/female) | Groups | Age | Appliance | T0 | T1 | T2 | T3 | T4 | Collection methods | Microbial analysis methods | Quality of the study |
|-----------|--------------|--------------------------|--------|-----|-----------|----|----|----|----|----|---------------------|---------------------------|--------------------|
| Lombardo et al. [33] | RCT | 20 (15 F/5 M) | 2 | G1: Mean: 19.3 years ± 3.6 months | CB vs. lingual braces | Before bonding | 4 weeks | 8 weeks | | Stimulated saliva collected by chewing paraffin gum for 5 min and expectorating into a sterile cup | Colonies were counted | B |
| Maret et al. [34] | Prospective study (case control) | 95 (56 F/39 M) | 2 | G1: Mean: 12–16 years | CB vs. control | Before bonding | 6 months | | | Salivary SM and LB | Dentocult® SM strips and Dentocult® LB method | C |
| Mattingly et al. [3] | Prospective study | 10 (6 M/4 F) | 1 | G1: Mean: 12–25 years | | | | | | | | |
| Miura et al. [35] | RCT | 40 | 2 | G1: 20 Fluoride-releasing elastomeric ligature ties vs. conventional elastomeric ligature ties | Fluoride-releasing elastomeric ligature ties vs. conventional elastomeric ligature ties | Before ligation | 7 days | 14 days | 28 days | Saliva and plaque samples. A sterilized curette was used to collect plaque samples from the area surrounding the ligature ties of the right UL, left UL, left L3, and right L5 | Number of SM CFU | B |
| Nalçaci et al. [36] | Prospective study | 46 (14 F/22 M) | 2 | G1: Mean: 11–16 years | SL braces vs. CB | Before bonding | 1 week | 5 weeks | | Microbial samples taken from the buccal surfaces of all bonded teeth | Number of colonies determined under a stereomicroscope | B |
| Ortu et al. [56] | Prospective study | 30 (15 M/15 F) | 3 | G1: Mean: 6–9 years | RPE vs. McNamara expander vs. controls | Before initiation of expansion therapy | 3 months | 6 months | | Whole stimulated saliva, stimulated with paraffin-based sticks | CFU of SM and LB | B |
| Pandis et al. [38] | RCT | 32 | 2 | Mean: 13.6 years | CB ligated with conventional elastomeric modules vs. SL braces | Before bonding | 2–3 months | | Collect saliva in the mouth and to expectorating into a chilled empty petri dish approximately 3 ml of saliva | Salivary SM and total bacteria were enumerated and analyzed after growth in culture | B |
| Reference         | Study design       | Sample size (male/female) | Groups | Age               | Appliance | T0          | T1          | T2          | T3          | T4          | Collection methods                                                                 |
|-------------------|--------------------|---------------------------|--------|-------------------|-----------|-------------|-------------|-------------|-------------|-------------|-----------------------------------------------------------------------------------|
| Paolantonio et al. [39] | Prospective study  | 24 (11 M/13 F)            | 1      | 18–22 years       | CB in one dental arch vs. control sites | Before bonding | 4 weeks    | 8 weeks    | 12 weeks (removal) | 4 weeks after removal | Agar plates examined for presence of As Definitive identification made on the basis of the methods Gram-stain, nitrate reduction, production of catalase, urine and indole, growth on MacConkey agar, and fermentation reactions to carbohydrates |
| Pejda et al. [40]  | RCT                | 38 (13 M/25 F)            | 2      | Mean: 14.6 years ± 2.0 months | SL braces vs. CB | Before bonding | 6 weeks    | 12 weeks   | 18 weeks   | Subgingival plaque samples were obtained at 18 weeks (T3). Subgingival plaque removed with a probe. Subgingival plaque collected with a sterile paper point from the periodontal sulcus. Sites: U6-L6 and distobuccal sites of U2-L2 in both dental arches |
| Pelleginti et al. [41] | RCT (split mouth)  | 14 (12 full appliance, 2 on maxillary arch only) | 1      | 11.7–17.2 years  | SL braces vs. CB with elastomeric ligatures | Before bonding | 1 week     | 5 weeks    | Plaque specimens collected from labial surfaces surrounding the brackets of U2 and L2 with a sterilized dental scaler. Saliva collection chewing gum-shaped paraffin wax tablet chewed for 1–5 min | Total oral Streptococci: mitis salivarius agar Bacterial count Determination of ATP-driven bioluminescence with the Bac-Titer Glo Microbial Cell Viability Assay Kit |
| Reference      | Study design   | Sample size (male/female) | Groups | Age                             | Appliance | T0                  | T1                  | T2                  | T3                  | T4                  | Collection methods                                                                 | Microbial analysis methods | Quality of the study |
|----------------|---------------|---------------------------|--------|---------------------------------|-----------|----------------------|----------------------|----------------------|----------------------|----------------------|-------------------------------------------------------------------------------------|-----------------------------|----------------------|
| Perinetti et al. [42] | Prospective study | 21 (11 F/10 M)            | 1      | 12–18 years                     | CB        | Before bonding        | 28 days             |                      |                      |                      | Subgingival plaque and GCF: three 30 standardized sterile paper strips inserted 1 mm into the gingival crevice. Mesial and distal tooth sites U3 test (DC), its contralateral (CC), and antagonist (AC) used as controls. CC included in the orthodontic appliance, but not subjected to the orthodontic force. AC free from any appliance. Aa colonization was determined by culture methods, while ALP and AST activities were evaluated. | Spectrophotometrically       | B                    |
| Peros et al. [43]     | Prospective study | 23                        | 1      | 12–17 years                     | CB + bands + wire ligatures | Before bonding        | 6 weeks             | 12 weeks            | 18 weeks            |                      | Chewed bilaterally a piece of paraffin wax. Cultura incubator and a QRT bacteria test kit for SM and LB. |                          | B                    |
| Ristic et al. [44]    | Prospective study | 32 (13 M/19 F)            | 1      | 12–18 years                     | CB + bands | TX: First appointment T0: 3 weeks before bonding | 1 month             | 3 months             | 6 months            |                      | Two sterile paper points in to the deepest part of gingival sulcus. Sites: mesio-vestibular points of subgingival sulcus of: U6 right, U1 left, and U4 left. If one was missing, adjacent tooth from the same group was used. Subculturing gram-stain, and identification tests of biochemical reactions: for identification of bacteria species. Colonies of bacteria were counted. |                          | B                    |
| Ristic et al. [45]    | Prospective study | 32 (13 M/19 F)            | 1      | 12–18 years                     | CB + bands | Before bonding        | 1 month             | 3 months             | 6 months            |                      | Two sterile paper points in to the deepest part of gingival sulcus. Sites: mesio-vestibular points of subgingival sulcus of: U6 right, U1 left, and U4 left. If one was missing, adjacent tooth from the same group was used. Subculturing gram-stain, and identification tests of biochemical reactions: used for identification of bacterial species. |                          | B                    |
| Reference            | Study design      | Sample size (male/female) | Groups | Age          | Appliance                                      | Collection time | Collection methods                                                                 | Microbial analysis methods                                      | Quality of the study |
|----------------------|-------------------|---------------------------|--------|--------------|-----------------------------------------------|-----------------|--------------------------------------------------------------------------------------|-------------------------------------------------------------------|----------------------|
| Sfondrini et al. [46]| RCT (split mouth) | 20 (6 M/14 F)             | 1      | Mean 23.8 years | Buccal and lingual braces (same braces) vs. control | Before bonding   | 1 day, 7 days, 30 days                                                               | Microbiological samples from the brackets and the teeth. Supragingival dental plaque sterile curettes | B                    |
| Shukla et al. [47]   | RCT               | 60                        | 1      | 15-25 years   | CB                                            | Before bonding   | 2 months, 3 months                                                                  | Counts of SM were determined by using Dentocult SM kit           | B                    |
| Shukla et al. [58]   | RCT               | 60                        | 1      | 13-18 years   | CB                                            | Before bonding   | 2 months, 3 months                                                                  | Counts of SM were determined by using Dentocult SM kit           | B                    |
| Sinclair et al. [4]  | RCT               | 13 (5 M/8 F)              | 1      | Mean 14 years ± 1 month | CB + bands                                    | Before bonding   | 1 year                                                                               | Mean counts for the triplicate plates of the five types of medium used | C                    |
| Sudarri et al. [48]  | Prospective study | 22 (12 F/10 M)            | 1      | Mean 25.09 years ± 4.36 months | CB + elastomeric ligatures                   | Before bonding   | 12 weeks                                                                              | PCR for SM and S. sobrinus                                       | C                    |
| Thornberg et al. [49]| Prospective study | 190 (47% M/ 53% F)        | 1      | 13.6 years    | CB                                            | Before bonding   | 6 months, 12 months, >12 months, ≤3 months after removal | Subgingival plaque samples: sterile paper points Sites: Mesial U5, distal U1, right, mesial L6 left, distal L1 left (if extracted mesial L5) | C                    |
| Topaloglu et al. [50]| Prospective study | 69 (31 F/38 M)            | 2      | 6-17 years    | CB vs removable appliance                      | Before appliance | 1 month, 3 months, 6 months                                                          | DNA probe technique                                               | C                    |
| Todorovic et al. [51]| Prospective study | 20 (8 M/12 F)             | 1      | Mean 12 years ± 1 month | CB                                             | Before bonding   | 4 weeks, 3 months, 5 months                                                           | PCR + HOMM                                                       | C                    |
| Turkshahman et al. [52]| RCT (split mouth) | 21 (12 F/9 M)             | 1      | (Two subgroups: G1: Elastomeric G2: Ligature wire) | CB + elastomeric rings vs ligature wires       | Before bonding   | 1 week, 5 weeks                                                                     | Microbial samples from labial surfaces of US                      | B                    |

(Continued)
| Reference       | Study design   | Sample size (male/female) | Groups | Age                  | Appliance                          | Collection time | Collection methods                                                                 | Microbial analysis methods                  | Quality of the study |
|----------------|----------------|---------------------------|--------|----------------------|------------------------------------|-----------------|--------------------------------------------------------------------------------------|---------------------------------------------|---------------------|
| Türköz et al.  | Prospective study | 24 (11 M/13 F)            | 1      | 14-20 years          | CB and thermoplastic retainers in the retention period | T0: 15 days, T1: 30 days, T2: 60 days | Split about 5 ml of saliva into 50 ml sterile tubes. Plaque samples collected with sterile swabs from gingival margin and enamel surface of each tooth at vestibule and palatal-lingual sides | Total viable SM and LB colonies were counted - means of CFUs per milliliter of volume (CFU/ml) | B                   |
| Uzuner et al.  | RCT            | 40 (29 F/11 M)            | 2      | 14-16 years          | CB + steel wire ligature vs. SL braces | Before bonding: 1 month | Microbial samples were collected from the stimulated saliva and the plaque from the labial surfaces of the U2-L2, immediately surrounding the orthodontic brackets with a dental scaler | To estimate the number of CFUs of SM and LB, Dentocult SM and LB kits were used | B                   |
| Van Gastel et al. | Prospective study | 24 (10 M/14 F)           | 2      | Mean: 14.6 years ± 1.1 month | Headgear + bands + CB vs. CB | G1: 18 weeks before G2, G2: Bonding time | Periapical absorbent strips into the sulcus for 30 s. The mesiobuccal and distobuccal sites of the U4 and U6 right sites were sampled. In the headgear group, U6 was banded, and U4 was bonded; the samples were analyzed separately | Total numbers of anaerobic and aerobic colony CFUs were counted. Pure cultures were identified by biochemical tests (including N-acetyl-D-glucosaminidase, α-glucosidase, α-galactosidase, α-fucosidase, esculine, indole, and trypsin activity) | B                   |
| Wichelhaus et al. | Prospective study | 11                       | 1      | Mean: 12.7 years      | CB | Before bonding: 4 weeks, 12 weeks | Plaque removed from dental surfaces using a sterile curette. Sites: incisors, premolars and molars | PCR – 13C urea breath tests for HP – Dentocult® SM – Dentocult® LB | C                   |
| Zheng et al.   | RCT            | 50 (23 M/27 F)            | 1      | Mean = 13.6 years    | CB | Before bonding: 1 month, 2 months, 3 months, 6 months | Gargle method | Samples cultured in CHROMagar Candida Identification | Different Candida strains identified based on the color of the colonies + PCR | C                   |
Table 3. Swedish council on technology assessment in health-care (SBU) criteria for grading assessed studies.

| Level | Evidence | Definition |
|-------|----------|------------|
| A     | Strong   | At least two studies assessed with level ‘A’ |
| B     | Moderate | One study with level ‘A’ and at least two studies with level ‘B’ |
| C     | Limited  | At least two studies with level ‘B’ |
| B     | Inconclusive | Fewer than two studies with level ‘B’ |

Table 4. Definitions of evidence level.

| Level | Evidence | Definition |
|-------|----------|------------|
| 1     | Strong   | At least two studies assessed with level ‘A’ |
| 2     | Moderate | One study with level ‘A’ and at least two studies with level ‘B’ |
| 3     | Limited  | At least two studies with level ‘B’ |
| 4     | Inconclusive | Fewer than two studies with level ‘B’ |

**CB**

Of the 29 articles that studied CB [3,4,10,12,13,19–23,26–30,32,34,35,38,41,44,46–48,50,51,54,44,57], the majority showed a significant increase in BOP and PI. Two studies [10,52] investigated the differences between the use of elastomeric or steel ligatures, revealing contradictory results on BOP and PI at different times. Ristic’s studies [44,45] highlighted that maximum values of PI and BOP were reached 3 months after appliance placement, followed by a decrease in these parameters 6 months after treatment began. Six studies assessed the increase of Candida at different times [12,13,22,23,56,57].

Twenty studies highlighted the increase of gram-positive bacteria, in particular *S. mutans* and *Lactobacillus* spp. [3,4,12,19–21,27–30,32,34,35,42,46,47,50,51,54,57]. Three studies [43,44,48] detected significant increases of gram-negative bacteria, respectively, at 3 and 6 months, followed by a decrease at 6 and 12 months. Ten studies [10,20,21,26,30,37,41,43,44,46] detected an increase in the percentage of gram-bacteria and *A. actinomyctemcomitans*. The study conducted by Alves de Souza et al. [10] revealed a significant increase in gram-species with the use of elastomeric rings (Table 5).

**Self-ligating braces**

Eight studies analyzed self-ligating braces (SLB) [9,14,24,37–40,54]. Two studies [14,40] revealed no differences for BOP and PI between SLB and CB, while Nalçac et al. and Uzuner et al. [54] demonstrated a worsening in SLB. Two studies considered the use of SLB with or without elastomeric rings, observing an increase in gram-concentration [24,38]. One other study [14] showed an increase of *S. mutans* and *Lactobacillus* spp. at 3 months with the use of SLB compared to controls. One study [41] showed less *S. mutans* with SLB compared to CB (Table 6).

**Lingual braces**

Four studies analyzed lingual braces (LB) [15,16,33,45] and three of these highlighted a worsening of PI and BOP [15,16,33]. Two studies [16,33] revealed an increase of *S. mutans* and *A. actinomyctemcomitans* after 4 weeks (Table 7).

**Removable appliances**

Six studies analyzed removable devices [11,17,18,31,49,52]. One study analyzed different interchangeable removable appliances [49], demonstrating an increase in both *S. mutans* and *Lactobacillus* spp.

The invisalign study, conducted by Levrini et al. [31], revealed lower values of PI, BOP, and bacterial component at 3 months for the invisalign group.

In the two studies with thermoplastic retainers, Türköz et al. [52] showed an increase of *S. mutans* and *Lactobacillus* spp. while Farhadian et al. [18] observed that the addition of silver nanoparticles reduced the levels of *S. mutans* after 7 weeks.

In one study [11], the use of space maintainers defined an increase in BOP in the number of bacteria and in Candida. Furthermore, D’Ercole et al. [17] pointed out that the use of sports mouth-guards produced an increase in BOP and PI (Table 8).

**Other appliances**

Two studies investigated other kinds of orthodontic appliances [25,56]: one fixed interceptive orthodontic appliance and one esthetic brace. In a study that analyzed fixed interceptive appliances, Ortu et al. [56] demonstrated an increase in *S. mutans* and *Lactobacillus* spp. (Table 9).

**Discussion**

The present systematic review agreed with the conclusions arrived at by Freitas et al. [5], which could be extended to any type of orthodontic appliance. The evidence of the selected sample was of medium-high level due to the lack of error of measurements analysis for the collection of material from oral sites. Though this lack of standardization may influence the outcomes, due to the difficulty in obtaining a high repeatability in this procedure, it would not
Table 5. Conventional braces results.

| Reference | PI | BOP | Microbiological analysis |
|-----------|----|-----|--------------------------|
| Alves et al. [10] | Elastomeric rings: Value (T0) = 37.72%; value (T1) = 63.72% | Elastomeric rings: Value (T0) = 4.28%; value (T1) = 12.28% | T0 steel ligatures-elastomeric rings: P(Aa) = 0.3173; P(Tf) = 0.1797; P(Pg) = /; P(Pi) = /; P(Pn) = 1.000 |
| | Steel ligatures: Value (T0) = 37.72%; value (T1) = 51.09% | Steel ligatures: Value (T0) = 3.86%; value (T1) = 6.71% | T0–T1 elastomeric rings: P(Aa) = 0.5637; P(Tf)<0.0001; P(Pg) = /; P(Pi) = 1.000; P(Pn)<0.0001 |
| | | | T0–T1 steel ligatures: P(Aa) = 0.5637; P(Tf) = 0.0003; P(Pg) = /; P(Pi) = /; P(Pn) = 0.0003 |
| Arab et al. [12] | Elastomeric rings: Value (T0) = 4.28%; value (T1) = 12.28% | Steel ligatures: Value (T0) = 3.86%; value (T1) = 6.71% |
| | Elastomeric rings: Value (T0) = 4.28%; value (T1) = 12.28% | Steel ligatures: Value (T0) = 3.86%; value (T1) = 6.71% |
| Arslan et al. [13] | Elastomeric rings: Value (T0) = 4.28%; value (T1) = 12.28% | Steel ligatures: Value (T0) = 3.86%; value (T1) = 6.71% |
| Forsberg et al. [19] | Elastomeric rings: Value (T0) = 4.28%; value (T1) = 12.28% | Steel ligatures: Value (T0) = 3.86%; value (T1) = 6.71% |
| Hågg et al. [22] | T0–T1–T2: P < 0.05 | | |
| Hernández-Solis et al. [23] | | | |
| Kim et al. [26] | Only significant values: T. forsythia: T2 vs. T3: U6: 0.013*; L6: 0.039* T2 vs. T4: U6: 0.002**; L1: 0.003**; L6: 0.012* T3 vs. T4: L1: 0.021* C. rectus: T1 vs. T2: U6: 0.007** P. nigrescens: T1 vs. T2: U6: 0.013*; L6: 0.022* SM CFU: G2 (control): mean – (SD)+ T0: 39–16; T1: 34–11 G1: mean – (SD)+ T0: 28–6; T1: 30–11 Pretest differences: P > 0.001 |
| | | | |
| Kupietzky et al. [27] | O’Leary’s plaque index: P = 0.061 | | |
| Lara-Carrillo et al. [28] | | | |

(Continued)
represent a major concern for the studies’ quality. In our sample, the use of orthodontic devices resulted in an increase in oral bacterial counts in patients, with significant differences between appliance type, depending on whether they were removable or not.
Previous studies have assessed the role of biomaterials in the regulation of the oral microbiota [58]. As stated by Antonelli et al. [59], the simplest surfaces for bacteria to colonize are hard ones as mucous membranes tend to scale off and, therefore, do not guarantee a stable adhesion. The only exception to this is the tongue, which is highly colonized even if it is a mucosal surface because of the irregular surfaces of papillae [60]. Consequently, the introduction of a biomaterial into this open system creates a further retentive surface on which bacterial species are able to reproduce and where there is an increased difficulty in maintaining oral hygiene [58]. As revealed by the Øilo and Bakken [58] literature review, the presence of biomaterials results in an increase in plaque and alterations in the oral microbiota.

Thus, on the basis of these assessments, it seems reasonable to state that the grade of bacterial colonization of the oral cavity is likely to be influenced by the type of biomaterial used. This factor may be critical in determining the relative success of implantation and the overall longevity of the biomaterial.

Table 5. (Continued).

| Reference          | PI | BOP | Microbiological analysis                                                                 |
|--------------------|----|-----|-----------------------------------------------------------------------------------------|
| Ristic et al. [44] | Incisors: T0: 0.898 ± 0.329; T1: 1.211 ± 0.278; T2: 1.250 ± 0.336; T3: 1.219 ± 0.275; Molars: T0: 0.625 ± 0.354; T1: 1.107 ± 0.219; T3: 1.070 ± 0.264 | (mean ± SD) | Difference between frequency of bacteria types compared between different recording periods on incisors, premolars, and molars: |
|                    | T2: 1.281 ± 0.310; T3: 1.318 ± 0.269; T1: 1.320 ± 0.586; T2: 1.336 ± 0.677; T3: 1.383 ± 0.453 | (mean ± SD) | Number determined in different periods of control: |
|                    | Premolars: T0: 0.547 ± 0.329; T1: 0.984 ± 0.126; T2: 1.055 ± 0.198; T3: 1.031 ± 0.123 | | Incisors: T0: 0.898 ± 0.329; T1: 1.211 ± 0.278; T2: 1.250 ± 0.336; T3: 1.219 ± 0.275; Molars: T0: 0.625 ± 0.354; T1: 1.107 ± 0.219; T3: 1.070 ± 0.264 |
|                    | Molars: T0: 0.227 ± 0.249; T1: 0.394 ± 0.358; T2: 0.602 ± 0.347; T3: 0.547 ± 0.367 | | T0–T1: P > 0.05; T0–T2: P < 0.01; T0–T3: P > 0.05; T1–T2: P > 0.05; T1–T3: P > 0.05; T2–T3: P > 0.05 |
| Ristic et al. [45] | Incisors: T0: 0.898 ± 0.329; T1: 1.211 ± 0.278; T2: 1.250 ± 0.336; T3: 1.219 ± 0.275; Molars: T0: 0.625 ± 0.354; T1: 1.107 ± 0.219; T3: 1.070 ± 0.264 | (mean ± SD) | Total bacterial count compared between different recording periods on incisors, premolars, and molars: |
|                    | Premolars: T0: 0.547 ± 0.329; T1: 0.984 ± 0.126; T2: 1.055 ± 0.198; T3: 1.031 ± 0.123 | (mean ± SD) | Incisors: T0: 0.898 ± 0.329; T1: 1.211 ± 0.278; T2: 1.250 ± 0.336; T3: 1.219 ± 0.275; Molars: T0: 0.625 ± 0.354; T1: 1.107 ± 0.219; T3: 1.070 ± 0.264 |
|                    | Molars: T0: 0.227 ± 0.249; T1: 0.394 ± 0.358; T2: 0.602 ± 0.347; T3: 0.547 ± 0.367 | | T0–T1: P > 0.05; T0–T2: P < 0.01; T0–T3: P > 0.05; T1–T2: P > 0.05; T1–T3: P > 0.05; T2–T3: P > 0.05 |
| Shukla et al. [47] | Plaque index: NS | | |
Table 5. (Continued).

| Reference | Plaque levels increase: NS | Prevalence of gingivitis at U1 increased from T0: 25% to T3: 74% | Microbiological analysis |
|-----------|----------------------------|---------------------------------------------------------------|--------------------------|
| Torlakovic et al. [51] | G1–G2: NS | T0–T1: P < 0.001; T0–T2: P < 0.001 | Statistical comparison of bacterial counts of the groups: |
| Turkkahraman et al. [52] | Bonded bracket plaque index: | T0 and T1: G1 = G2 T2: Significantly more bleeding in G2 | Total bacteria: NS |
| Van Gastel et al. [20] | Banded bracket plaque index: | | Anaerobe lactobacilli: NS |
| Wichelhaus et al. [55] | API: | | Longitudinal changes in bacterial counts of bonded: |
| Zheng et al. [56] | | | Total bacteria: |

/ : dental site negative; *P<0.05.

Colonization related to orthodontic appliances is affected by the energy and roughness of the appliance surfaces, as well as their design and dimensions. This may be a key factor in efficiently performing hygiene procedures [58].

Another significant variable for microbiota alterations is the amount of time the appliance is worn in the oral cavity, with removable appliances having significantly less impact on oral bacteria than fixed appliances [61].
The quantitative alteration of the oral microbiota is related to an increase in clinical parameters, PI and BOP, which are risk indicators for oral pathologies [62].

Together with the quantitative change, there is also a qualitative variation; indeed, there is an increase in gram-positive and gram-negative more aggressive bacteria, such as: S. mutans and...
**Table 7. Lingual braces results.**

| Reference          | Buccal sites: | BOP | Microbiological analysis |
|--------------------|---------------|-----|-------------------------|
| Demling et al.     | Labial:       |     |                         |
| [15]               | G1:           |     |                         |
|                    | T0: 0.1 ± 0.2; T1: 1.0 ± 0.2 |     |                         |
|                    | Buccal sites: |     |                         |
|                    | T0: 2.2 ± 1.9; T1: 5.6 ± 3.1 |     |                         |
|                    | Labial:       |     |                         |
|                    | T0: 0.1 ± 0.2; T1: 1.0 ± 0.2 |     |                         |
|                    | Buccal sites: |     |                         |
|                    | T0: 0.1 ± 0.2; T1: 1.2 ± 1.1 |     |                         |
|                    | Labial:       |     |                         |
|                    | T0: 0.2 ± 0.5; T1: 0.0 ± 0.1 |     |                         |
|                    | Buccal sites: |     |                         |
|                    | T0: 1.9 ± 2.0; T1: 13.5 ± 13.6; P: 0.184 |     |                         |
|                    | Labial:       |     |                         |
|                    | T0: 0.1 ± 0.1; T1: 0.1 ± 0.2 |     |                         |
|                    | Buccal sites: |     |                         |
|                    | T0: 2.5 ± 2.2; T1: 22.2 ± 18.9; P: 0.608 |     |                         |
|                    | Labial:       |     |                         |
|                    | T0: 18.1 ± 17.5; T1: 12.9 ± 16.7; P: 0.101 |     |                         |
|                    | Buccal sites: |     |                         |
|                    | T0: 23.4 ± 22.5; T1: 46.2 ± 23.5; P: 0.001 |     |                         |
|                    | Labial:       |     |                         |
|                    | T0: 3.3 ± 3.3; T1: 1.0 ± 0.7; P: 0.001 |     |                         |
| Lombardo et al. [33] | G2:           |     |                         |
|                    | T0: 0.47 ± 0.18; T1: 0.56 ± 0.15; T2: 0.59 ± 0.16 |     |                         |
|                    | G1:           |     |                         |
|                    | T0: 0.3 ± 0.2; T1: 0.4 ± 0.17; T2: 0.52 ± 0.25; T2: 0.4 ± 0.20 |     |                         |
|                    | T0–T1: P < 0.05; T1–T2: NS; T0–T2: P < 0.5 |     |                         |
|                    | NS differences (P > 0.05) in the different groups at different times |     |                         |
| Sfondrini et al. [46] | SM            |     |                         |
|                    | G2:           |     |                         |
|                    | T0: 0.18 ± 0.13; T1: 0.22 ± 0.07; T2: 0.29 ± 0.19 |     |                         |
|                    | Total CFU/P value: |     |                         |
|                    | SM            |     |                         |
|                    | G1:           |     |                         |
|                    | T0: 0.31 ± 0.21; T1: 0.45 ± 0.17; T2: 0.33 ± 0.13 |     |                         |
|                    | T0–T1: P < 0.05; T1–T2: P < 0.01 |     |                         |
|                    | NS            |     |                         |
|                    | G1:           |     |                         |
|                    | T0: 0.18 ± 0.13; T1: 0.22 ± 0.07; T2: 0.29 ± 0.19 |     |                         |
|                    | L-control: 4.64E + 7/0.41 |     |                         |
|                    | V-L: 4.65E + 6/0.68; V-control: 3.00E + 5/0.07; P: 0.001 |     |                         |
|                    | L-control: 4.64E + 7/0.41 |     |                         |
|                    | NS            |     |                         |

Pi: Plaque index; BOP: bleeding on probing.

The *Lactobacillus* spp. (gram-positive) and *P. gingivalis*, *T. forsythia*, and *T. denticola* (gram-negative); and these bacteria are closely associated with, respectively, enamel and dentin pathologies (e.g. demineralizations or caries) and with periodontal disease [63]. Recent papers have highlighted the complexity of periodontal disease etiology, with a special focus on the identity of bacteria which are responsible for this pathology [64–66]. Thus, authors have stated that the presence alone of specific microbial species seems insufficient in causing gingivitis and periodontal disease, and that the change in biofilm equilibrium is another key factor in the development of these diseases [64–66]. Oral microbiota alterations registered in orthodontic patients appear to be consistent with the modifications occurring in patients with poor oral hygiene presenting gingivitis and/or periodontal diseases. In addition, orthodontic devices could represent a direct risk factor for periodontal diseases as they are often related to an increase in periodontopathogenic species [24,43,44,48]. However, it seems reasonable to state that the susceptibility of each subject, as well as other factors that may alter the biofilm balance, may play a key role in determining the entity of periodontal sequelae.

Even though changes in the microbial system involve all types of orthodontic appliance, more rapid modifications occur during fixed orthodontic treatment. These alterations may be recorded even 1 month after the beginning of treatment and may lead to a decrease in patients’ periodontal health perception [41]. Even so, as stated by Perinetti et al. [41], the role of subgingival bacteria in periodontal modifications needs to be evaluated together with the action of enzymes activated in response to the stimuli of orthodontic forces.

If it is true that all appliances increase the bacterial component, it is also the case that mobile devices make minor changes as they are removable and can be completely cleaned, resulting in better oral hygiene minimizing retentive artifacts. It should also be emphasized that, of these appliances, the use of mouthguards is limited to a small population and they are carried only for limited periods of time, involving a less pathogenic effect.

Less devastating results from changes in the oral microbiota emerged from studies on functional appliances and on aligners, which are used up to 22 h a day [61]. So, it seems more
important to be able to remove the appliance and wash both it and the teeth rather than the length of time the device is worn.

In view of the changes in microbiota that occurred with the introduction of biomaterials into the oral cavity, and more specifically of the orthodontic devices, it would be appropriate for patients undergoing dedicated hygiene protocols to keep the oral bacterial charge under control and then to reduce the risk of the carious process and periodontal disease, as evidenced by various authors [2,67,68].

Conclusions

- The overall evidence quality level was moderate-to-high, thus significant conclusions could be drawn.
- Orthodontic appliances significantly influence the oral microbiota, independent of appliance type.
- Significant alterations of the microbiota were registered 1 month after the start of treatment.
- Removable appliances had less impact on oral bacteria than fixed ones.
- Personalized professional and daily hygiene protocols are recommended for orthodontic patients from the beginning of treatment.

Table 8. Removable appliances results.

| Reference            | PI   | BOP   | Microbiological analysis                          |
|----------------------|------|-------|--------------------------------------------------|
| Arik et al. [11]     | G1:  |       | T0:                                             |
|                      |      |       | Mean Candida:                                    |
|                      |      |       | P(G1) = 0.68; P(G2) = 0.16                      |
|                      | G2:  |       | Total Candida:                                   |
|                      |      |       | P(G1) = 0.47; P(G2) = 0.19                      |
|                      |      |       | T2:                                             |
|                      |      |       | Mean Candida:                                    |
|                      |      |       | P(G1) = 0.003; P(G2) = 0.12                     |
|                      |      |       | Total Candida:                                   |
|                      |      |       | P(G1) = 0.01; P(G2) = 0.11                      |
|                      |      |       | T3:                                             |
|                      |      |       | Mean Candida:                                    |
|                      |      |       | P(G1) = 0.00; P(G2) = 0.04                      |
|                      |      |       | T0:                                             |
|                      |      |       | Mean Candida:                                    |
|                      |      |       | P(G1) = 0.00; P(G2) = 0.07                      |
|                      |      |       | T0:                                             |
|                      |      |       | Mean Candida:                                    |
|                      |      |       | P(G1) = 0.00; P(G2) = 0.07                      |
| Ortu et al. [57]     |      |       | T0:                                             |
|                      |      |       | Mean Candida:                                    |
|                      |      |       | P(G1) = 0.00; P(G2) = 0.04                      |
|                      |      |       | T0:                                             |
|                      |      |       | Mean Candida:                                    |
|                      |      |       | P(G1) = 0.00; P(G2) = 0.07                      |
|                      |      |       | T0:                                             |
|                      |      |       | Mean Candida:                                    |
|                      |      |       | P(G1) = 0.00; P(G2) = 0.04                      |
|                      |      |       | T0:                                             |
|                      |      |       | Mean Candida:                                    |
|                      |      |       | P(G1) = 0.00; P(G2) = 0.07                      |
|                      |      |       | T0:                                             |
|                      |      |       | Mean Candida:                                    |
|                      |      |       | P(G1) = 0.00; P(G2) = 0.07                      |
|                      |      |       | T0:                                             |
|                      |      |       | Mean Candida:                                    |
|                      |      |       | P(G1) = 0.00; P(G2) = 0.07                      |
|                      |      |       | T0:                                             |
|                      |      |       | Mean Candida:                                    |
|                      |      |       | P(G1) = 0.00; P(G2) = 0.07                      |
|                      |      |       | T0:                                             |
|                      |      |       | Mean Candida:                                    |
|                      |      |       | P(G1) = 0.00; P(G2) = 0.07                      |

Table 9. Other appliances results.

| Reference            | PI   | BOP | Microbiological analysis |
|----------------------|------|-----|--------------------------|
| Jurela et al. [25]   | SM   | BOP | NS                       |
| Ortu et al. [57]     | Group 1: | T1-T2: | NS; LB (T1-T2): NS; SM (T1-T2): NS |
|                      |       |     | Statistical significant: |
|                      |       |     | LB (T1-T2): P = 0.011; SM (T1-T2): P = 0.005; |
|                      |       |     | LB (T2-T0): P = 0.007; SM (T2-T0): P = 0.006; |
|                      |       |     | G2: LB (T1-T2): NS |
|                      |       |     | Statistical significant: |
|                      |       |     | LB (T2-T0): P = 0.006; SM (T2-T0): P = 0.004; |
|                      |       |     | LB (T1-T0): P = 0.01; SM (T1-T0): P = 0.006; SM (T1-T2): P = 0.03 |
| PI: Plaque index; BOP: bleeding on probing. |
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