The Microbiologic Profile Associated with Peri-Implantitis in Humans: A Systematic Review

Mia Rakic, DDS, PhD1/Maria Gabriella Grusovin, DDS2/Luigi Canullo, DDS, PhD3

Purpose: To qualitatively investigate the microbiologic profile in peri-implantitis by systematically reviewing the published literature on peri-implant infection. Materials and Methods: Searches of the US National Institutes of Health free digital archives of the biomedical and life sciences journal literature (PubMed) and The Cochrane Library of the Cochrane Collaboration (CENTRAL), as well as a hand search of other literature, were conducted to identify articles potentially relevant for the review. Randomized clinical trials, prospective cohort studies, longitudinal studies, case-control studies, and cross-sectional studies in humans reporting microbiologic findings in patients with diagnosed peri-implantitis were considered eligible for this review. Screening, data extraction, and quality assessment were conducted independently and in duplicate. Results: Twenty-one articles were eligible for inclusion in this review. Early studies focused on the identification of target periopathogens, whereas more recent studies used advanced molecular techniques for comprehensive overview of the peri-implantitis–associated microbiome. In summary, the microbiologic profile in peri-implantitis (1) is complex and variable, (2) consists of gram-negative anaerobic periopathogens and opportunistic microorganisms in almost the same ratio, (3) is frequently associated with the Epstein-Barr virus and nonsaccharolytic anaerobic gram-positive rods, (4) is not so strictly associated with Staphylococcus aureus, and (5) is different from that of periodontitis. A meta-analysis could not be performed because of the heterogeneity of the reviewed studies. Conclusion: Although a comparison of the published results was limited because of the inhomogeneity of the studies, it is clear that the microbiologic profile of peri-implantitis consists of aggressive and resistant microorganisms and is distinct from that of periodontitis. It seems that the quantitative characteristics of the microflora cohabitants represent the key determinant of disease, rather than the qualitative composition, which is very similar in healthy and peri-implantitis states. INT J ORAL MAXILLOFAC IMPLANTS 2016;31:359–368. doi: 10.11607/jomi.4150

Keywords: bacteria, microbiota, peri-implantitis, periodontitis

Peri-implantitis is a late complication of oral implants characterized by inflammatory bone loss.1 The central pathologic process in peri-implantitis is inflammatory osteoclastogenesis, which could be triggered by infection, lesions of the peri-implant attachment, excessive biomechanical stress, and/or biocorrosion.2 However, infection remains the major cause of peri-implantitis, and it is considered that all other factors sooner or later act in conjunction with infection.2–4 Although peri-implantitis was once considered a counterpart of periodontitis at implant sites, recent studies have indicated that peri-implant pathology seems to be more complex and differs from periodontal disease pathology.5 This complexity relates primarily to the structural specificities of peri-implant tissues and to the implants themselves. Some studies have suggested that the lack of periodontal ligament and cementum makes the peri-implant tissues more susceptible to infection and trauma and leads to fast progression of disease.6–11 Furthermore, the implant surface oxide layer (so-called “ceramic-like” layer), which is the major factor of titanium implant biocompatibility, requires certain physicochemical conditions to maintain its stability and, hence, implant biocompatibility. Related to this, the anaerobic peri-implant infection, which creates an acid microenvironment full of reactive host-derived factors (such as free radicals), is considered a physicochemical threat to the stability
of the oxide layer that could increase inflammatory bone loss. For these reasons, knowledge of the microbiologic profile associated with peri-implantitis (which would guide infection control and supportive periodontal therapy measures) seems to be one of the factors that is essential for the success of implant treatment, particularly in subjects with a history of periodontitis. Despite this great necessity, the microbiologic profile of peri-implantitis has not yet been clearly defined.13–15

The objective of this review was to qualitatively estimate the microbiologic profile associated with peri-implantitis in humans through a review of the literature.

MATERIALS AND METHODS

This systematic review complies with the PRISMA guidelines (Preferred Reporting items for Systematic Reviews and Meta-Analyses; www.prisma-statement.org).

Study Protocol and Criteria

The protocol was designed to answer the following question: “What are the characteristics of the microbiologic profile in patients with peri-implantitis?” Included were randomized clinical trials, prospective cohort studies, case-control studies, and cross-sectional studies in humans reporting microbiologic findings in patients diagnosed with peri-implantitis. Peri-implantitis was defined as the radiographic presence of bone loss ≥ 2 mm since the time of prosthetic replacement, positive bleeding on probing, and probing depth ≥ 5 mm. Only studies published in English were included. Excluded were in vitro and animal studies and studies of blade implants.

Furthermore, the following PECO (population, exposure, comparison, outcome) definitions were considered.

- Population. Studies had to include systemically healthy patients with at least one implant affected by peri-implantitis; microbiologic findings from affected sites had to be provided.
- Exposure. Peri-implantitis was the exposure considered for evaluation.
- Comparison. The specific comparisons investigated were either differences throughout the course of peri-implantitis or differences between peri-implantitis and peri-mucositis, healthy peri-implant tissues, or periodontitis.
- Outcome measures. The primary outcome variable was microbiologic status (total flora, presence of certain bacterial pathogens, and percentages and proportions of flora of certain bacterial pathogens).

Search Strategy

Searches of the U.S. National Institutes of Health free digital archives of biomedical and life sciences journal literature (PubMed) and The Cochrane Library of the Cochrane Collaboration (CENTRAL), as well as a hand search of other literature, were conducted to identify articles of potential relevance. The search included articles accepted for publication up to August 2014. Previously published review articles on similar topics were also analyzed to assess potentially relevant publications. The following key words were used for this purpose: periimplantitis OR peri-implantitis OR peri-implantitis OR peri-implantitis OR peri-implant AND infection OR bacteria OR microorganism OR biofilm OR plaque.

Quality Assessment

Quality assurance was developed according to Khan et al16 via independent screening by two reviewers, resolution of disagreement by consensus, discarding of studies when consensus was not achieved, and data extraction in duplicate.

Data Extraction and Synthesis

Two independent reviewers (MR, LC) analyzed titles and abstracts in the first stage of screening. Irrelevant articles were discarded. Then, the full texts of the articles considered to be potentially relevant for the review were read to determine whether the studies fulfilled the predetermined inclusion criteria. Any disagreement regarding eligibility of the articles was individually resolved between the reviewers. Data were collated into evidence tables and grouped according to the microbiologic analyses that were performed: (1) evaluation of target pathogens or (2) evaluation of the entire microbiome. Furthermore, the extracted data were stratified and expressed in chronologic order according to the publication date. Synthesis of the data was performed based on the evidence tables alone, and the data were further interpreted in relation to the performed study design (cross-sectional, case-control, or longitudinal). Meta-analysis was not carried out because of the marked heterogeneity of many aspects of the included studies.

RESULTS

The initial search of the literature up to June 2014 yielded 631 potentially suitable papers. Following exclusion of reviews, animal and in vitro studies, and studies that inappropriately identified peri-implantitis, 21 publications remained fully eligible for this review. The κ value for interreviewer agreement for study inclusion was 0.93 for titles and abstracts and 1.00 for full-text articles, indicating strong agreement. The major findings...
of the reviewed articles were sorted into two tables according to the type of microbiologic analysis performed. Table 1 covers the studies that evaluated the target pathogens,17-31 and Table 2 includes studies that evaluated the entire microbiome.32-37

**Target Pathogens of Peri-implantitis**

Fifteen studies evaluated target pathogens using cultures, checkerboard hybridization, polymerase chain reaction (PCR), or DNA probes. Most studies were cross-sectional and case-control studies; only three were longitudinal studies.

In the case-control study evaluating the microbiologic profiles of peri-implantitis and healthy peri-implant tissues, distinct differences were observed between peri-implantitis and clinically healthy implants.17 Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Prevotella intermedia, and Prevotella nigrescens were identified in 60%, while *Staphylococcus epidermidis*, enterics, and *Candida albicans* were seen in 55% of cases. *P intermedia* and *P nigrescens* were the most common organisms found in peri-implantitis, while *Enterobacter* and *Klebsiella* were the most common of the enterics. However, *A actinomycetemcomitans* was the microorganism most significantly associated with peri-implantitis, whereas *A actinomycetemcomitans* and *P gingivalis* were seen in only one patient with healthy tissues. Another cross-sectional study21 of the microbiologic profiles of 213 participants with healthy implants, peri-implant mucositis, and peri-implantitis demonstrated no significant differences between these conditions. However, peri-implant pockets with the deepest probing depth were correlated with levels of *Eikenella corrodens*, *Fusobacterium nucleatum* sp *vincentii*, *P gingivalis*, and *Micromonas micros*. Moreover, the case-control study that evaluated the microbiologic profiles of patients with peri-implantitis and healthy subjects did not report higher mean counts of *P gingivalis*, *Treponema denticola*, or *Tannerella forsythia* in both supramucosal and submucosal samples in peri-implantitis patients, whereas there was no significant difference between supra- and submucosal specimens originating from the same site.22 Another cross-sectional study evaluated periopathogens in patients with peri-implantitis, peri-implant mucositis, and healthy peri-implant tissues and reported high levels of *A actinomycetemcomitans*, *P gingivalis*, *P intermedia*, *T forsythia*, and *T denticola* in peri-implantitis.19 Another cross-sectional study evaluated 40 different bacterial species among patients with peri-implantitis, peri-implant mucositis, and healthy implants and indicated that *Actinomyces gerencseriae* was present in lower mean counts and *T forsythia* was present in higher mean counts in peri-implantitis than in implants that were diagnosed with peri-implant mucositis and in healthy implants, whereas *Capnocytophaga ochracea* was higher in mucositis than in the other groups.23 It was also observed that *P gingivalis* showed the highest levels in peri-implantitis as opposed to the healthy implants, at which red-complex periopathogens were found in very low levels. Furthermore, a cross-sectional study investigated microflora in 15 cases of peri-implantitis using cultures and established an association among *T forsythia*, *Campylobacter* sp, and *Parvimonas micra* with peri-implantitis.24 Additionally, a positive correlation between pain and the presence of *P micra*, *Fusobacterium*, and *Eubacterium* sp was reported. A more recent cross-sectional study estimated the frequency of *Campylobacter rectus*, *P gingivalis*, *T forsythia*, *P intermedia*, *T denticola*, and *A actinomycetemcomitans* between equivalent peri-implant and periodontal conditions; it showed that the occurrence of investigated bacteria was generally higher in teeth than in implants.25 The frequency of all bacteria except *P intermedia* was significantly higher in peri-implantitis compared to healthy implants, while *P gingivalis* and red-complex species occurred more frequently in peri-implantitis than in peri-implant mucositis. *P gingivalis* and *A actinomycetemcomitans* were similarly distributed between periodontitis and peri-implantitis, but the occurrence of all other species was higher in periodontitis than in peri-implantitis. Moreover, a case-control study evaluated 78 different bacterial species among peri-implantitis and healthy implant samples and showed a bacterial load of *T forsythia*, *P gingivalis*, *Treponema socranskii*, *Staphylococcus aureus*, *Streptococcus intermedius*, *Streptococcus mitis*, and *Haemophilus influenzae* that was increased by about four times in peri-implantitis when compared to healthy implants.26 Following comprehensive statistical analysis, it was suggested that this cluster of bacteria, including *T forsythia* and *S aureus*, is associated with peri-implantitis.

Three studies evaluated the association of periopathogenic viruses with peri-implantitis.27,28,31 One study27 analyzed the presence of human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV) within different peri-implant conditions and showed a high prevalence of HCMV and EBV in the subgingival plaque of peri-implantitis sites. The same group of authors estimated the prevalence of different genotypes of HCMV and EBV in subgingival plaque samples from peri-implantitis, peri-implant mucositis, and healthy implants and reported a high prevalence of HCMV-2 and EBV-1 in peri-implantitis.28 Furthermore, an estimation of both periopathogenic bacteria and viruses in saliva and subgingival samples from healthy implant and peri-implantitis sites in 23 patients demonstrated higher counts of EBV, CMV, *T denticola*, and *T forsythia* in peri-implantitis.31 Evaluation of these microorganisms as susceptibility factors for peri-implantitis showed
that peri-implantitis was 14.2 and 3 times more likely to harbor EBV than healthy implants and saliva, whereas the odds ratios for \textit{T. denticola} and \textit{T. forsythia} were 6.79 and 3 times higher as well, respectively; thus, EBV was suggested as a potential risk factor for peri-implantitis. In the three longitudinal studies considered in this review,\textsuperscript{18,20,25} researchers evaluated periopathogens as well as some opportunistic microorganisms and the responses to different treatment approaches. One study\textsuperscript{18} included 30 implants affected by peri-implantitis and evaluated the treatment effect of tetracycline. At baseline, high frequencies of \textit{C. rectus}, \textit{T. forsythia}, \textit{Fusobacterium} \textit{sp}, and \textit{P. intermedia/nigrescens} and low frequencies of \textit{A. actinomycetemcomitans}, \textit{P. gingivalis},

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of cases</th>
<th>Study</th>
<th>Sampling method</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leonhardt et al\textsuperscript{17} (1999)</td>
<td>37 peri-implantitis, 51 healthy implants</td>
<td>Case-control</td>
<td>PP</td>
<td>Culture</td>
</tr>
<tr>
<td>Mombelli et al\textsuperscript{18} (2001)</td>
<td>25 peri-implantitis</td>
<td>Longitudinal</td>
<td>PP</td>
<td>Culture, DFM</td>
</tr>
<tr>
<td>Hultin et al\textsuperscript{19} (2002)</td>
<td>17 patients; 45 peri-implantitis, 53 healthy implants</td>
<td>Cross-sectional</td>
<td>PP</td>
<td>DNA probe analysis</td>
</tr>
<tr>
<td>Leonhardt et al\textsuperscript{20} (2003)</td>
<td>9 peri-implantitis</td>
<td>Longitudinal</td>
<td>PP</td>
<td>Culture</td>
</tr>
<tr>
<td>Renvert et al\textsuperscript{21} (2007)</td>
<td>31 peri-implantitis, 127 peri-implant mucositis, 55 healthy implants</td>
<td>Cross-sectional</td>
<td>PP</td>
<td>DCH</td>
</tr>
<tr>
<td>Shibli et al\textsuperscript{22} (2008)</td>
<td>22 peri-implantitis, 22 healthy implants</td>
<td>Case-control</td>
<td>CT</td>
<td>DCH</td>
</tr>
<tr>
<td>Maximo et al\textsuperscript{23} (2009)</td>
<td>13 peri-implantitis, 12 peri-implant mucositis, 10 healthy implants</td>
<td>Cross-sectional</td>
<td>CT</td>
<td>DCH</td>
</tr>
<tr>
<td>Tabanella et al\textsuperscript{24} (2009)</td>
<td>15 peri-implantitis, 15 healthy implants</td>
<td>Case-control (split-mouth)</td>
<td>PP</td>
<td>Culture</td>
</tr>
<tr>
<td>Persson et al\textsuperscript{25} (2010)</td>
<td>31 peri-implantitis</td>
<td>Randomized longitudinal</td>
<td>PP</td>
<td>Expanded DCH assay</td>
</tr>
<tr>
<td>Casado et al\textsuperscript{26} (2011)</td>
<td>10 peri-implantitis, 10 peri-implant mucositis, 10 healthy implants</td>
<td>Cross-sectional</td>
<td>PP</td>
<td>PCR</td>
</tr>
<tr>
<td>Jankovic et al\textsuperscript{27} (2011)</td>
<td>20 peri-implantitis, 18 mucositis, 18 healthy implants</td>
<td>Cross-sectional</td>
<td>PP</td>
<td>Qualitative PCR</td>
</tr>
<tr>
<td>Jankovic et al\textsuperscript{28} (2011)</td>
<td>35 peri-implantitis, 30 peri-implant mucositis, 30 healthy implants</td>
<td>Cross-sectional</td>
<td>PP</td>
<td>Qualitative PCR</td>
</tr>
<tr>
<td>Cortelli et al\textsuperscript{29} (2013)</td>
<td>53 healthy implants, 53 periodontally healthy, 50 peri-implant mucositis, 50 peri-implantitis</td>
<td>Cross-sectional</td>
<td>CT</td>
<td>PCR</td>
</tr>
<tr>
<td>Persson and Renvert\textsuperscript{30} (2013)</td>
<td>166 peri-implantitis, 47 healthy implants</td>
<td>Case-control</td>
<td>PP</td>
<td>DCH</td>
</tr>
<tr>
<td>Verdugo et al\textsuperscript{31} (2014)</td>
<td>23 peri-implantitis, 23 healthy implants</td>
<td>Case-control (split-mouth)</td>
<td>PP</td>
<td>Qualitative PCR for periopathogens, Quantitative PCR for EBV and CMV</td>
</tr>
</tbody>
</table>
### Microbiologic findings in peri-implantitis

<table>
<thead>
<tr>
<th>Distinct bacterial profile between peri-implantitis and healthy implants. PG, AA, PI, PN in 60%, Staphylococcus epidermidis, enterics, and Candida albicans in 55%. PI and PN the most common microorganisms, while AA was significantly associated with peri-implantitis. Staphylococci, enterics, and yeasts more frequent in peri-implantitis than in periodontitis. Similar profile between healthy implants and periodontium.</th>
<th>Brånemark, Nobel Biocare</th>
</tr>
</thead>
<tbody>
<tr>
<td>45%: CR, FU, PI/PN; TF 36% PI/PN, Fusobacterium sp, BF, and CR reduced levels M1, M3, and M6 following treatment.</td>
<td>ITI</td>
</tr>
<tr>
<td>75%–100% implants: AA, FN, PN, PI; 50%–75% implants: PG, PM, CR, EC</td>
<td>14 Brånemark, 3 ITI</td>
</tr>
<tr>
<td>7 patients: PI/PN; 6 patients: AA; 3 patients: enterics; 1 patient: PG, SA. PI/PN and enterics persisted 6 mo, 1 y, 5 y posttreatment.</td>
<td>Nobel Biocare</td>
</tr>
<tr>
<td>EC, FB sp vincentii, PG, and MM were correlated with the deepest pocket depths.</td>
<td>Brånemark, Nobel Biocare</td>
</tr>
<tr>
<td>Higher counts of PG, TD, TF in peri-implantitis. Supra- and subgingival profiles were not substantially different.</td>
<td>Brånemark-like</td>
</tr>
<tr>
<td>AG lower counts, TF higher counts in peri-implantitis compared to mucositis and healthy implants. PG was at the highest levels in peri-implantitis. PM, TF, PG, and TD from TS significantly reduced 3 mo posttreatment in peri-implantitis.</td>
<td>Brånemark</td>
</tr>
<tr>
<td>9 implants: FU, TF; 7 implants: CR, PM; 5 implants: PG, PI</td>
<td>11 Brånemark, 4 3i</td>
</tr>
<tr>
<td>Baseline: AA (serotype b), FN sp, HP, Staphylococcus sp, and TF. 30 minutes following curettage: reduced counts of AA, Lactobacillus acidophilus, Streptococcus anginosus, and Veillonella parvula. Baseline and 6 mo: no differences in bacterial counts.</td>
<td>No data</td>
</tr>
<tr>
<td>AA, PG, PI, TD, and TF identified in all conditions</td>
<td>No data</td>
</tr>
<tr>
<td>HCMV 13 (65%), EBV in 9 (45%) in peri-implantitis</td>
<td>Predominantly Nobel Biocare</td>
</tr>
<tr>
<td>HCMV-2 in 53.3% and EBV-1 in 46.6% peri-implantitis</td>
<td>Predominantly Nobel Biocare</td>
</tr>
<tr>
<td>CR, PG, TF, TD, and AA higher in peri-implantitis than in healthy implants. PG and red-complex species higher in peri-implantitis compared to mucositis. PG and AA similar between periodontitis and peri-implantitis; CR, TF, PI, TD higher in periodontitis.</td>
<td>Nobel Biocare</td>
</tr>
<tr>
<td>TF, PG, TS, SA, SI, SM, and HI 4× increased total load in peri-implantitis and suggested a cluster of bacteria, including TF and SA, as related to peri-implantitis.</td>
<td>No data</td>
</tr>
<tr>
<td>Higher counts of EBV, CMV, TD, and TF in peri-implantitis. Peri-implantitis 14.2 and 3 times more likely to harbor EBV than healthy implants and saliva, while odds ratios for TD and TF were 6.79 and 3 times higher than in healthy sites.</td>
<td>No data</td>
</tr>
</tbody>
</table>

and *E. corrodens* were seen. Another study evaluated the effect of open-flap debridement in conjunction with systemic antibiotics against target microorganisms. At baseline, six sites were positive for *A. actinomycetemcomitans*, seven for *P. intermedia* and *P. nigrescens*, one for *P. gingivalis*, one for *S. aureus*, and three for both *Escherichia coli* and *Enterobacter cloacae*. At 6 months and 1 year posttreatment, *P. intermedia/P. nigrescens* and enterics persisted; after 5 years, *P. intermedia/P. nigrescens* had reached a peak, as they were now present in eight patients (versus seven patients at baseline). Furthermore, in a study that evaluated treatment outcomes in patients with peri-implantitis after two different kinds of mechanical debridement (curettes/
Great differences were observed between healthy implants compared to peri-implantitis. FN, the microbial composition of peri-implantitis is more diverse than that of periodontitis. The periodontal microbiome demonstrated significantly higher diversity than the implant. Distinct bacterial species were identified in peri-implantitis, 57 negative species and was more diverse than in healthy implants.35

The microorganisms detected only in peri-implantitis were Chloroflexi, Tenericutes, and Synergistetes sp, P. micra, Peptostreptococcus stomatis, Pseudoramibacter alactolyticus, and Solobacterium moorei, while A. actinomycetemcomitans and P. gingivalis were present in low levels. Hence, it was concluded that most of the microbiota in peri-implantitis consisted of gram-negative species and was more diverse than in healthy implants and periodontitis. Another cross-sectional study33 analyzed subgingival/submucosal plaque samples from 40 participants with periodontitis, peri-implantitis, and periodontal and peri-implant health using 16S pyrosequencing and reported lower levels of Prevotella and Leptotrichia sp and higher levels of Actinomyces, Peptococcus, and Campylobacter sp, non-mutans streptococci, Butyrivibrio sp, and Streptococcus mutans in peri-implantitis when compared to healthy implants.33 A comparison of peri-implantitis and periodontitis demonstrated lower levels of Prevotella sp, non-mutans streptococci, and Lactobacillus, Selenomonas, Leptotrichia, and Actinomyces sp and higher levels of Peptococcus, Mycoplasma, Eubacterium, Campylobacter, and Butyriviribrio sp, S mutans, and Treponema sp in peri-implantitis. Hence, the authors concluded that the microbiomes of periodontitis and peri-implantitis differ significantly. A similar study demonstrated that the microbiologic profile was more diverse in peri-implantitis than in periodontitis.34 Fusobacterium sp and Streptococcus sp were predominant in both disorders, while P. micra was detected only in peri-implantitis. This study outlined the

Table 2  Major Microbiologic Findings Obtained by Metagenomic and Metatranscriptomic Analysis of the Entire Microbiome in Peri-implantitis

<table>
<thead>
<tr>
<th>Authors</th>
<th>N cases</th>
<th>Study</th>
<th>Sampling method</th>
<th>Identification</th>
</tr>
</thead>
</table>
| Koyanagi et al32 (2010) | 3 peri-implantitis  
3 healthy implants  
3 periodontitis | Cross-sectional | PP | 16S rRNA gene sequencing |
| Kumar et al33 (2012)  | 10 peri-implantitis  
10 healthy implants  
10 periodontally healthy  
10 periodontitis | Cross-sectional | PP | 16S pyrosequencing |
| Koyanagi et al34 (2013) | 6 peri-implantitis,  
6 periodontitis | Cross-sectional | PP | 16S rRNA gene sequencing |
| Tamura et al35 (2013)  | 15 peri-implantitis  
15 healthy implants | Case-control | PP | Culture +  
16S DNA gene sequencing |
| Dabdoub et al36 (2013) | 20 peri-implantitis  
13 gingivitis  
12 periodontitis | Cross-sectional | PP | 16S rRNA gene sequencing |
| da Silva et al37 (2010) | 10 peri-implantitis  
10 healthy implants | Case-control | CT | 16S rRNA gene sequencing |

Sampling: CT = curette; PP = paper points.
Microbiologic methods: DCH = DNA–DNA checkerboard hybridization; DFM = dark-field microscopy.
Bacteria: AA = Aggregatibacter actinomycetemcomitans; AG = Actinomyces gerencseriae; BF = Bacteroides forsythus; CA = Capnocytophaga sp; CMV = Cytomegalovirus; CR = Campylobacter rectus; EBV = Epstein-Barr virus; EC = Eikenella corrodens; FN = Fusobacterium nucleatum; FU = Fusobacterium sp; MM = Micromonas micros; PG = Porphyromonas gingivalis; PI = Prevotella intermedia; PM = Parvimonas micros; PN = Prevotella nigrescens; SA = Staphylococcus aureus; SM = Streptococcus mutans; TF = Tannerella forsythia; TD = Treponema denticola; TS = Treponema socranski.

Microbiome of Peri-implantitis

In a cross-sectional study that analyzed the microbiologic profile using a sequencing technique, 77 bacterial species were identified in peri-implantitis, 57 in periodontitis, and 12 around healthy implants.32 The microorganisms detected only in peri-implantitis were Chloroflexi, Tenericutes, and Synergistetes sp, P. micra, Peptostreptococcus stomatis, Pseudoramibacter alactolyticus, and Solobacterium moorei, while A. actinomycetemcomitans and P. gingivalis were present in low levels. Hence, it was concluded that most of the microbiota in peri-implantitis consisted of gram-negative species and was more diverse than in healthy implants and periodontitis. Another cross-sectional study33 analyzed subgingival/submucosal plaque samples from 40 participants with periodontitis, peri-implantitis, and periodontal and peri-implant health using 16S pyrosequencing and reported lower levels of Prevotella and Leptotrichia sp and higher levels of Actinomyces, Peptococcus, and Campylobacter sp, non-mutans streptococci, Butyriviribrio sp, and Streptococcus mutans in peri-implantitis when compared to healthy implants.33 A comparison of peri-implantitis and periodontitis demonstrated lower levels of Prevotella sp, non-mutans streptococci, and Lactobacillus, Selenomonas, Leptotrichia, and Actinomyces sp and higher levels of Peptococcus, Mycoplasma, Eubacterium, Campylobacter, and Butyriviribrio sp, S mutans, and Treponema sp in peri-implantitis. Hence, the authors concluded that the microbiomes of periodontitis and peri-implantitis differ significantly. A similar study demonstrated that the microbiologic profile was more diverse in peri-implantitis than in periodontitis.34 Fusobacterium sp and Streptococcus sp were predominant in both disorders, while P. micra was detected only in peri-implantitis. This study outlined the
higher prevalence of periopathogens and more complex microflora in peri-implantitis compared to periodontitis. Furthermore, in another case-control study, microbiologic specimens obtained from healthy implant sites and peri-implantitis were investigated; the results showed 10-fold higher mean colony-forming units in peri-implantitis.\(^{35}\) The predominant species in peri-implantitis sites were Streptococcus and Eubacterium, while the predominant species around healthy implants were Streptococcus, Veillonella, and Actinomyces. Although Streptococcus was common in both groups, the prevalence of bacterial species was completely different between the investigated groups. Sixty-nine different bacterial species were identified in peri-implantitis sites, and predominant bacterial species were Eubacterium nodatum, E brachy, E saphenum, Filifactor alocis, Slackia exigua, Parascardovia denticolens, P intermedia, F nucleatum, P gingivalis, Centipeda periodontii, and P micro; the number of species at healthy implants was 53. Based on the significantly higher prevalence of nonsaccharolytic anaerobic gram-positive rods in peri-implantitis and compared to healthy implant sites, where these bacteria were almost undetectable, it was suggested that this group of bacteria could play an important role in peri-implantitis. Furthermore, a cross-sectional study was performed\(^ {36}\) to profile peri-implant and periodontal microflora in healthy and diseased conditions using a sequencing method; it revealed that peri-implant and periodontal microbiomes are distinct ecosystems and indicated a more diverse profile in periodontitis. Members of the genera Staphylococcus and Treponema were significantly associated with peri-implantitis.\(^ {36}\) A case-control study that estimated microbiomes of healthy implants and peri-implantitis showed different profiles for peri-implantitis and healthy implants; peri-implantitis was associated with more periopathogenic bacterial species than healthy implants.\(^ {37}\) Higher mean proportions of F nucleatum, Campylobacter gracilis, Dialister invisus, Streptococcus sp, Eubacterium infirmum, Filifactor alocis, and Mitsuokella sp presented a higher mean proportion, while Veillonella dispar, Streptococcus mitis, Actinomyces meyeri, Granulicatella diadens showed lower mean proportions in the peri-implantitis sites than in healthy implants.
DISCUSSION

As pointed out in a previous review of this issue, early studies focused on comparisons of the microbiologic profiles in peri-implantitis and chronic periodontitis using routine culture techniques, hybridization, and PCR to evaluate target periopathogenic bacteria, ie, the so-called periopathogens. In brief, these studies showed that the qualitative profile of periopathogens was similar between healthy and peri-implantitis–affected implants, whereas the quantitative composition of periopathogens was the distinguishing factor between health and disease. Furthermore, an analysis of the periopathogen profiles of healthy versus diseased implants and corresponding conditions in teeth revealed an increased frequency of periopathogens in teeth. Generally, investigations of periopathogens were unable to establish a clear microbiologic profile associated with peri-implantitis, and it was accepted that the microbiologic and immunopathologic profiles of peri-implantitis and periodontitis differ. Therefore, more recent studies have been directed toward comprehensive analyses of the microbiome in peri-implantitis. Advanced molecular techniques, such as sequencing methods, render a comprehensive overview of phylogenetic and taxonomic bacterial diversity and indirectly compensate for the limitations of cultures and lack of reactivity of conventional biochemical tests that are typical for a number of fastidious anaerobic microorganisms. For the aforementioned reasons, during the last 4 years the newer sequencing approaches have been widely used to profile the peri-implantitis microflora and offer new insights. Microbiologic screens employing sequencing techniques have shown the following: (1) peri-implantitis harbored mainly gram-negative species, and an association of certain periopathogens with peri-implantitis was confirmed; (2) nonsaccharolytic bacteria are associated with peri-implantitis; (3) S aureus is not strictly present in peri-implantitis; (4) a different and more diverse microflora is present in peri-implantitis than in healthy implants; (5) the microbiomes in peri-implantitis and periodontitis are different ecosystems. In fact, these metagenomic and metatranscriptomic techniques revealed more diverse microbiologic profiles in both periodontitis and peri-implantitis than previously thought, and in general it has been suggested that the role of periopathogens must be re-interpreted. When considering the reported results as a whole, the peri-implantitis microflora consists of periopathogens and opportunistic bacteria in almost the same measure. The lack of conclusive evidence about the impact of periopathogens on the peri-implantitis microflora could be possibly explained by the recently reported “keystone pathogen” hypothesis of periodontal infection. This hypothesis indicates that similar bacteria are present in both healthy and diseased conditions, while the changes in bacterial proportions represent the key pathologic determinant. In addition, it is suggested that the most important role of periopathogens is the conversion of a symbiotic ecosystem into a dysbiotic one. Translated to peri-implantitis, it seems that periopathogens provide permissive conditions for the overgrowth of opportunistic bacteria, thus orchestrating their conversion from physiologically harmless to pathological members of microflora. In this regard, an evaluation of opportunistic bacteria is the subject of recent studies that focused primarily on the identification of these bacteria. One study quantitatively evaluated opportunistic bacteria together with periopathogens and estimated their risk rate. It showed that, of 78 analyzed bacteria, a cluster of four opportunistic bacteria (S aureus, S intermedius, S mitis, and H influenzae) and three periopathogens (T forsythia, P gingivalis, and T socranskii) are associated with peri-implantitis. This additionally supports the theory that opportunistic bacteria and their quantitative content represent the important determinants of the microbiologic profile in peri-implantitis. Considering the reviewed studies as a whole, there is a high rate of inhomogeneity in their protocols that significantly reduces the applicability of the reported results. The use of several different diagnostic protocols contributed to inhomogeneity in the expression of microbiologic outcome variables (total bacterial counts, frequencies, proportions, loads, etc); thus, comparisons of the findings between groups remain limited. In general, the reviewed studies were performed on relatively small samples (never more than 50 peri-implantitis cases, and, on average, 20 peri-implantitis cases) and provided very heterogenous observational findings that render between-study comparisons difficult. Hence, despite the great efforts of the implant research community, most of the reported results are observational and descriptive, such that it is almost impossible to determine meaningful patterns concerning the microbiologic profile of peri-implantitis. Furthermore, earlier studies mostly evaluated the qualitative and quantitative profiles of periopathogens, whereas more recent studies revealed the implications of other pathogens, which remain merely identified, while their quantitative profiles and pathologic mechanisms have not yet been clearly established. Moreover, the reported results originated predominantly from case-control and cross-sectional studies (only 3 of 21 were longitudinal studies), although it seems that changes in bacterial counts represent the key determinant of peri-implantitis microflora. Therefore, future research in this area should be oriented toward the quantitative assessment of peri-implant microflora cohabitants.
in well-designed prospective studies. Since it is obvious that the peri-implantitis microbiologic profile is complex and determined by numerous members with complex interrelationships, advanced statistical tools for risk estimation should be considered in future research as well.

When considering the microbiologic profile from a clinical standpoint, peri-implantitis is associated with aggressive and resistant bacterial strains and viruses; therefore, mechanical anti-infective treatment usually fails to reduce or eliminate periopathogens without auxiliary antimicrobial therapy. Furthermore, the opportunistic bacteria characteristic of peri-implantitis are highly resistant and do not respond to some antibiotics that are routinely administered in periodontology, such as metronidazole. Additionally, it was previously reported that the implant-abutment connection could harbor a reservoir of bacteria in both healthy and diseased implants, based on the "circular model" of bacterial contamination. Therefore, it seems that the contaminated implant surface represents a reservoir of periopathogens with the ability to firmly adhere to both biotic and abiotic systems. This could possibly explain the better treatment outcome in patients in whom surface decontamination was performed compared to the outcome following routine mechanical treatment with curettes and ultrasound devices. Related to this, microbiologic contamination of the implant surface represents an intrinsic characteristic of peri-implant pathology that can interrupt the stability of the implant surface oxide layer and stimulate further detrimental processes. Finally, effective infection control through targeted anti-infective approaches, implant surface decontamination, and supportive periodontal therapy remains crucial treatment for peri-implantitis.

CONCLUSIONS

Considering the reviewed studies as a whole, the microbiologic profile in peri-implantitis: (1) is complex and variable, (2) consists of gram-negative anaerobic periopathogens and opportunistic microorganisms in almost the same ratio, (3) is frequently associated with Epstein-Barr virus and nonsaccharolytic anaerobic gram-positive rods, (4) is not so strictly associated with Staphylococcus aureus, and (5) is different from that of periodontitis. It seems that the presence of titanium creates a distinct microenvironment, and as a consequence, the microbiologic profile in peri-implantitis remains different from that of periodontitis. Furthermore, microorganisms that constitute the microbiologic profile in peri-implantitis have the potential to disrupt the stability of the implant surface layer and, hence, might contribute to a pathologic interface with the implant surface.

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REFERENCES


