BIOCELL
2023 47(2): 431-439

Correlations among core species corresponding to the clinical staging of periodontitis

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Key words: Quantitative polymerase chain reaction, Bacterial correlation, Bacterial counts

Abstract: The correlation between microbiota plays a vital role in the progression of periodontal disease. This study investigated the in situ interaction networks between periodontal pathogens in periodontal and peri-implant disease. We used quantitative real-time polymerase chain reaction and Pearson's correlation coefficients to quantify the copy numbers and correlations of four oral core species—*Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Streptococcus gordonii*—from 80 subgingival sites (healthy and with periodontitis or gingivitis) in patients with periodontitis, and 68 subgingival sites (healthy and with periodontitis, gingivitis, peri-implantitis, or peri-implant mucositis) in patients with implants. The highest bacterial counts were observed for *Porphyromonas gingivalis* and *Prevotella intermedia* at all the sites. Within the same cohorts, the bacterial loads were greater at diseased sites than at healthy sites. Bacterial counts did not differ among clinical sites in the same group ($P > 0.05$) but differed between periodontitis and peri-implant mucositis sites in the two groups. *Porphyromonas gingivalis*, *F. nucleatum*, and *Prevotella intermedia* had strong correlations at gingivitis and healthy sites and moderate correlations at periodontitis sites in patients with periodontitis. In patients with implants, *Prevotella intermedia*, *F. nucleatum*, and *S. gordonii* had strong correlations only at peri-implantitis sites. Also, based on metagenomic analysis, *F. nucleatum* and *Prevotella intermedia* were significantly correlated at the subgingival plaque in peri-implantitis and periodontitis samples. Our results suggest that variations in microbe-microbe interactions in subgingival plaque reflect changes in the progression of periodontal disease, providing a new perspective for understanding the mechanisms of periodontitis and peri-implantitis.

Introduction

Periodontal diseases include a range of conditions, from gingivitis to periodontitis, and gingivitis precedes periodontitis. If not treated properly, they eventually lead to tooth loss and even increase the risk of systemic problems (Slots, 2017). Implant-supported reconstruction is an ideal treatment for dental defects caused by severe periodontitis. While if there is no effective maintenance, peri-implant mucositis and peri-implantitis may occur. In peri-implant disease, collagen fibers are non-attached and parallel to the implant surface instead of being perpendicularly arranged from bone to cementum in periodontal disease (Meffert, 1996). Meanwhile, peri-implantitis has a more severe loss of bone than periodontitis because of the different microbiomes and higher microbial diversity in implant sites (Rokaya *et al.*, 2020). All these dental conditions are biofilm-mediated diseases. Oral biofilms are sophisticated structures created by the sequential and ordered interplay of multiple oral bacteria (Kolenbrander *et al.*, 2002). They are recognized as etiologic agents in caries or periodontal/peri-implant disease, which are a result of dysbiosis among plaque biofilm, host, and local microenvironment. Meanwhile, to maintain host-bacteria homeostasis, there is a need for a comprehensive understanding of interactions among microbial species and how they interact to initiate disease.

In one clinical study, Ritz (1967) reported that the dental plaque/biofilm formation due to the deposition of salivary

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Received: 15 June 2022; Accepted: 08 August 2022

Doi: 10.32604/biocell.2023.024948

proteins on the gum line, followed by accumulation of *Streptococcal* species, which covered nearly 90% of the dental biofilm after 2-day formation. As the plaque formation continues, Gram-positive aerobic *Streptococcal* species mix Gram-positive and Gram-negative anaerobic mcirobiota, including *streptococci* sp., *Actinomyces* sp., *Veillonella* sp., *Fusobacteria* sp., etc. (Moore *et al.*, 1987). Some research focused only on cataloging the differences in bacterial community members among the healthy and those having gingivitis and periodontitis (Lourenço *et al.*, 2014; Park *et al.*, 2015; Lu *et al.*, 2021). Although the correlation between changes in the structure and function of microbial communities in plaque biofilm and periodontal conditions are strongly implicated, these analyses overlooked that gingivitis and periodontitis are not independent but successive stages of the same disease process (Jeffcoat and Reddy, 1991; Shaw *et al.*, 2016). Later, Nowicki *et al.* (2018) analyzed the transition of dental conditions from healthy to having gingivitis and identified genes associated with early disease. Nemoto *et al.* (2021) described a health-to-periodontitis microbiome shift and identified core taxa associated with periodontal disease progression, and Huang *et al.* (2021) characterized temporal dynamics of the microbiome in gingivitis-to-periodontitis transition. Yu *et al.* (2019) reported that the composition of the submucosal and subgingival microbiota at periodontitis and peri-implantitis sites was similar in subjects in the same cohort. Nevertheless, these studies did not compare patterns of the bacterial interplay between the periodontal and peri-implant habitats, and the information gap between how the microbial interactions change during disease progression still remains.

To understand whether gingivitis and periodontitis represent a continuum, it would be of interest to evaluate the presence and levels of periodontitis-associated species in gingivitis and health and *vice versa*. Above all, the conditions involve interaction among microbiota rather than a single bacterium. The species associated with health appear to be species with demonstrated roles during early biofilm colonization in disease sites, such as *Streptococcus* spp. (Abusleme *et al.*, 2021). *Streptococcus gordonii* is a commensal bacteria of the oral cavity and pioneer colonizer during the formation of the dental plaque/biofilm (Nairn *et al.*, 2020), which are also critical microbiota related to biofilm formation. *Fusobacterium nucleatum*, the "bridge" in the oral biofilm development due to its ability to co-aggregate with representatives of all initial and late colonizers (Kolenbrander *et al.*, 2010), was identified as one of the "gingivitis-driver" species in a gingivitis model (Huang *et al.*, 2014), in addition to over-expression of virulence-related genes (Nowicki *et al.*, 2018). *Porphyromonas gingivalis*, and *Prevotella intermedia*, as late colonizers, are associated with periodontitis and peri-implantitis (Colombo and Tanner, 2019). So, the interactive relationship between microbiota during biofilm formation plays an important role in disease progression.

In this study, we collected subgingival plaque samples from periodontal healthy, gingivitis, and periodontitis sites in the same cohort and healthy peri-implant, peri-mucositis, and peri-implantitis sites in peri-implantitis patients, representing different stages of periodontal disease progression. Also, we selected *S. gordonii*, *Prevotella intermedia*, *F. nucleatum*, and *Porphyromonas gingivalis* as representative of different stages of subgingival plaque formation (Kolenbrander *et al.*, 2010) to assess their correlation to disease progression.

Materials and Methods

Subject enrollment and sampling

Patients with chronic periodontitis were recruited from the Department of Periodontology, Peking University School and Hospital of Stomatology, from December 2013 to May 2015 (Zhang *et al.*, 2017). The inclusion criteria were: good general health and no pregnancy, age 18–65 years (male or female), no antibiotic use in the past three months, and no periodontal therapy in the past year. Subjects who had any systemic condition that could affect the progress of periodontal disease were excluded.

Thirty-four patients with peri-implantitis were recruited from The Second Dental Center, Peking University School, and Hospital of Stomatology, between 2014 and 2017. The inclusion criteria were: good general health and no pregnancy, age 18–65 years, and partial edentulism due to severe periodontitis. Each patient had received at least one dental implant (Straumann, Basel, Switzerland) and one year of systemic periodontal treatment. The subjects had no other oral or systemic disease and had not taken antibiotics for at least three months before sampling.

Two professional dentists examined the periodontal and peri-implant conditions of the participants. Inclusion criteria of gingivitis and peri-implant mucositis were made based on the occurrence of bleeding and the absence of evidence of bone loss. Inclusion criteria of periodontitis and peri-implantitis were made based on the presence of bleeding and/or suppuration on gentle probing, PPD ≥ 5 mm, and attachment loss >5 mm (Berglundh *et al.*, 2018). Diagnostic criteria for the healthy conditions and healthy implants were the absence of inflammation, bleeding, and suppuration on gentle probing, the absence of evidence of bone loss, and the absence of an increase in PPD compared with the previous examination. Eighty subgingival sites (healthy and with periodontitis or gingivitis) in patients with periodontitis and 68 subgingival sites (healthy and with periodontitis, gingivitis, peri-implantitis, or peri-implant mucositis) in patients with implants were collected. The institutional review board of Peking University School and Hospital of Stomatology (Beijing, China) approved the study protocol (No. PKUSSIRB-2012063).

Sampling and DNA extraction

All patients were untreated with periodontal therapy. Before sampling, the patients rinsed their mouths with purified water, and then supragingival plaque and saliva around the sampling position (teeth and implants) were removed with sterile cotton pellets. The subgingival plaque in periodontal pockets was collected with sterile paper points for 10 s. Samples were subsequently placed in 1.5-mL centrifuge tubes with 1 mL TE buffer (20 mM Tris and 2 mM ethylenediaminetetraacetic acid; pH 7.4), transferred to a central laboratory, and frozen at −80°C.

Bacterial genomic DNA was extracted using QIAamp DNA mini kits (Qiagen, Valencia, CA, USA) according to

TABLE 1

The primers of the four target species

Primers	Strain ATCC number	Sequences(5′-3′)	References
Porphyromonas gingivalis	33277	TACCCATCGTCGCCTTGGT CGGACTAAAACCGCATACACTTG	Zhuang *et al.* (2016)
Prevotella intermedia	25611	AATACCCGATGTTGTCCACA TTAGCCGGTCCTTATTCGAA	Maeda *et al.* (2003)
Fusobacterium nucleatum	25586	CGCAGAAGGTGAAAGTCCTGTAT TGGTCCTCACTGATTCACACAGA	Suzuki *et al.* (2004)
Streptococcus gordonii	10558	TGTACCCCGTATCGTTCCTGTG AAAGACTGGAGTTGCAATGTGAATA	Park and Kook (2013)

the manufacturer's protocol. The quality and concentration were tested using the Nanodorp8000 device (Thermo Fisher Scientific, Wilmington, DE, USA). High-quality DNA with an optical density $(OD)_{260}/_{280}$ ratio of 1.8–2.0 and concentration ≥10 ng/μL was used for further analysis.

Bacterial loads of core species

The bacterial loads of four core species (*Porphyromonas gingivalis* W83, *F. nucleatum* ATCC 25586, *Prevotella intermedia* ATCC 15033, and *S. gordonii* ATCC 10558) in plaque were determined by quantitative polymerase chain reaction (qPCR). *Porphyromonas gingivalis*, *F. nucleatum*, and *Prevotella intermedia* were cultured in a brain heart infusion medium supplemented with hemin (5 mg/L) and vitamin K (1 mg/mL) at 37°C under anaerobic conditions, and *S. gordonii* was cultured in a brain heart infusion medium at 37°C in an atmosphere of 5% CO_2 and 95% air. Genomic DNA was extracted from the four selected standard strains to quantify the bacterial loads in clinical samples. Then, the standard quantified DNA was subjected to serial tenfold dilution from 10 ng to 10 fg for the plotting of standard curves. The clinical DNA samples and standard bacterial DNA were used as templates for PCR in a reaction volume of 20 μL containing 10 μL Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 0.5 μL of each primer (10 nM), and 2 μL DNA template. The amplification conditions were 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and at the four species-specific annealing temperatures for 60 s. Amplification was performed on an ABI QuantStudio real-time PCR system (Thermo Fisher Scientific). The four species-specific primers are listed in Table 1 (Zhuang *et al.*, 2016; Maeda *et al.*, 2003; Suzuki *et al.*, 2004; Park and Kook, 2013). All samples were run in triplicate.

Correlation analysis of four species based on metagenomic sequencing

We selected three metagenome data (PRJNA552294, PRJNA230363, PRJDB6966) derived from the subgingival samples collected from periodontitis patients, healthy people, and peri-implantitis patients based on whole genome sequencing (WGS). The first two data were obtained after sequencing on the ILLUMINA Hiseq platform, and the last data (PRJDB6966) was obtained after sequencing on the ILLUMINA Miseq platform. All the reads were subject to quality control as follows: containing less than 3% N bases and more than 50% bases with high quality (>3). The high-quality reads mapped to the human genome (hg19) were removed using the Kneaddata pipeline (http://huttenhower.sph.harvard.edu/kneaddata) with default parameters. Then, all the clean reads were aligned to the NCBI bacteria and Human Oral Microbiome Database using Kraken2 (Wood *et al.*, 2019) with default parameters. The correlation analysis was visualized using Spearman software. $P < 0.05$ was considered statistically significant.

Data analysis

The bacterial copy numbers were log-10 -transformed for analysis. Statistical analyses were performed using SPSS software Version 25 (SPSS Inc., Chicago, IL, USA), and $P < 0.05$ was taken to indicate significance. Correlations were assessed using Pearson's correlation coefficient and the Kruskal–Wallis rank-sum test. The strength of correlation between two bacteria was classified using *r*-values (0–0.19, very weak; 0.2–0.39, weak; 0.40–0.59, moderate; 0.6–0.79, strong; 0.8–1, very strong) (Ruengsomwong *et al.*, 2016).

Results

Bacterial loads of the four core species at different sites

A total of 80 plaque samples were collected from distinct subgingival sites (58 with periodontitis, 16 with gingivitis, and six healthy) in 43 patients with periodontitis, and 68 plaque samples were collected from distinct subgingival/submucosal sites (27 with periodontitis, seven with gingivitis, five with peri-implantitis, 20 with peri-implant mucositis, and nine with healthy implants) in patients with peri-implantitis.

The log-10–transformed counts of the four target species at each clinical sampling site from the two cohorts (cohort 1, patients with periodontitis; cohort 2, patients with peri-implantitis) are illustrated in Fig. 1. Also, total bacterial loads did not differ within or between the samples from the two cohorts (data not shown). With regard to each target species, no significant difference in the bacterial count was detected within or among different clinical sites in the same cohort.

The bacterial load of *Prevotella intermedia* was similar in all samples. The bacterial load of *Porphyromonas gingivalis* was significantly lower at healthy tooth sites in cohort 1

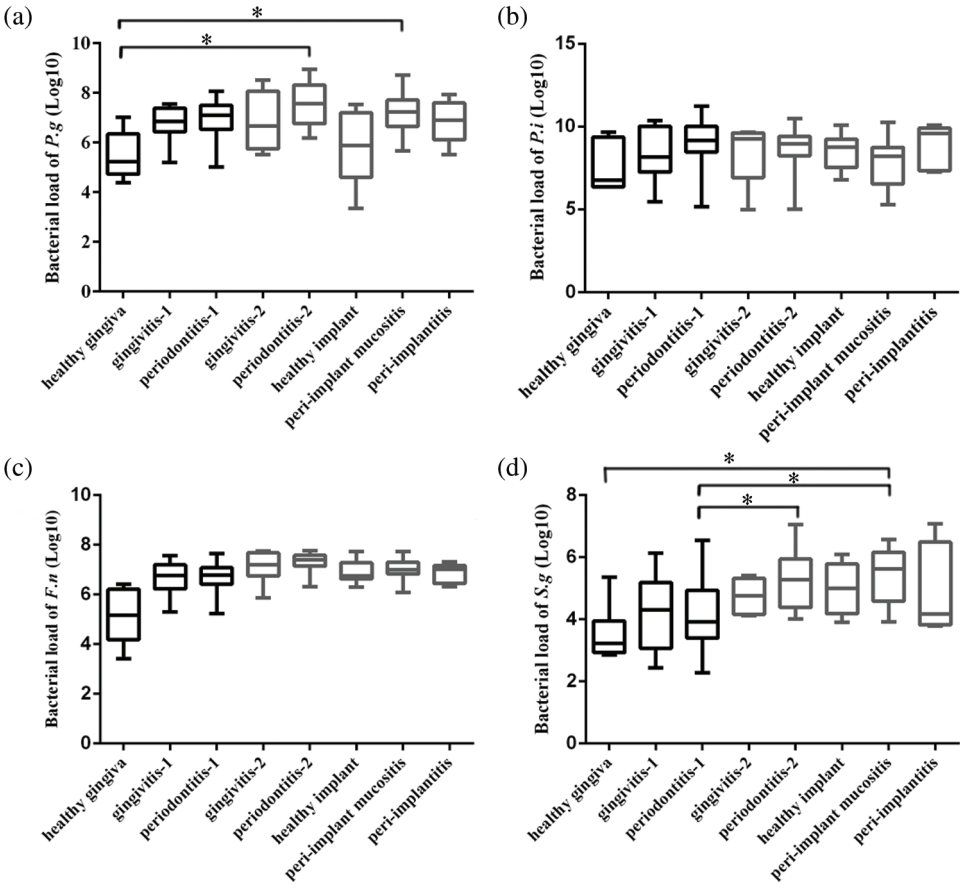

FIGURE 1. Bacterial loads of single species in the different clinical sites from patients with periodontitis and peri-implantitis. These findings were based on the qPCR results. All the tests were conducted in triplicate and the species were tested by the Kruskal-Wallis rank sum test. A significant difference was set at $P < 0.05$ with "*" marked. The black bold boxes represent sites from patients with periodontitis, and the gray boxes represent sites from patients with peri-implantitis.

than at sites of periodontitis and peri-implant mucositis in cohort 2. The bacterial load of *S. gordonii* differed significantly between periodontitis sites in cohort 1 and periodontitis and peri-implant mucositis sites in cohort 2. The same difference was also observed between healthy sites in cohort 1 and peri-implant mucositis sites in cohort 2. The bacterial load of *F. nucleatum* was greater at gingivitis and periodontitis sites in cohort 1 than that at periodontitis sites in cohort 2. In addition, the *F. nucleatum* bacterial load was greater at healthy sites in subjects with periodontitis than at periodontitis, gingivitis, and peri-implant mucositis sites in cohort 2.

Bacterial loads of the four core species at the same sites in subjects with periodontitis and those with peri-implantitis

In the same cohorts, the bacterial loads were greater at diseased sites than at healthy sites, but the differences were not significant. At periodontitis sites, the log-10–transformed bacterial loads of *F. nucleatum* and *S. gordonii* were significantly greater in subjects with periodontitis than in subjects with implants. *Prevotella intermedia* showed the greatest bacterial load at all sampling sites, and *S. gordonii* had the smallest bacterial load, except at peri-implantitis sites in cohort 2.

At peri-implantitis and healthy implant sites from cohort 2, loads of *Prevotella intermedia* and *S. gordonii* differed significantly. At periodontitis and gingivitis sites in the two cohorts, loads of these bacteria, but not those of *F. nucleatum* and *Porphyromonas gingivalis*, differed significantly. The results also revealed a significant difference for *S. gordonii* relative to the other three bacteria at peri-implant mucositis sites. At healthy sites in cohort 1, differences were significant for *Prevotella intermedia* and *Porphyromonas gingivalis*/*S. gordonii* (Fig. 2).

Correlations among the four core species in different sites from peri-implantitis and periodontitis patients

According to the abundance analysis, we observed a positively correlated network among *Porphyromonas gingivalis*, *F. nucleatum*, and *Prevotella intermedia* in the subgingival microbiota at clinically healthy (unaffected) and diseased periodontal sites in cohort 1. A weak positive correlation was found between *S. gordonii* and *F. nucleatum* at periodontitis sites. In cohort 2, *F. nucleatum* and *Prevotella intermedia* showed a strong correlation at peri-implant mucositis, peri-implantitis, and healthy implant sites; a moderate correlation was detected between *F. nucleatum* and *Porphyromonas gingivalis/Prevotella intermedia* at periodontitis sites. A positive correlation network among *S. gordonii*, *F. nucleatum*, and *Prevotella intermedia* was established only in the peri-implantitis sample group. In the peri-implant healthy and peri-implant mucositis groups, no correlation was found between *S. gordonii* and *F. nucleatum*/*Prevotella intermedia* (Fig. 3).

Correlation analysis among the four core species based on metagenomic data

Also, we downloaded data from 92 cases of periodontitis and 24 peri-implantitis to conduct metagenomic analysis; the results showed a significant correlation between *F. nucleatum* and

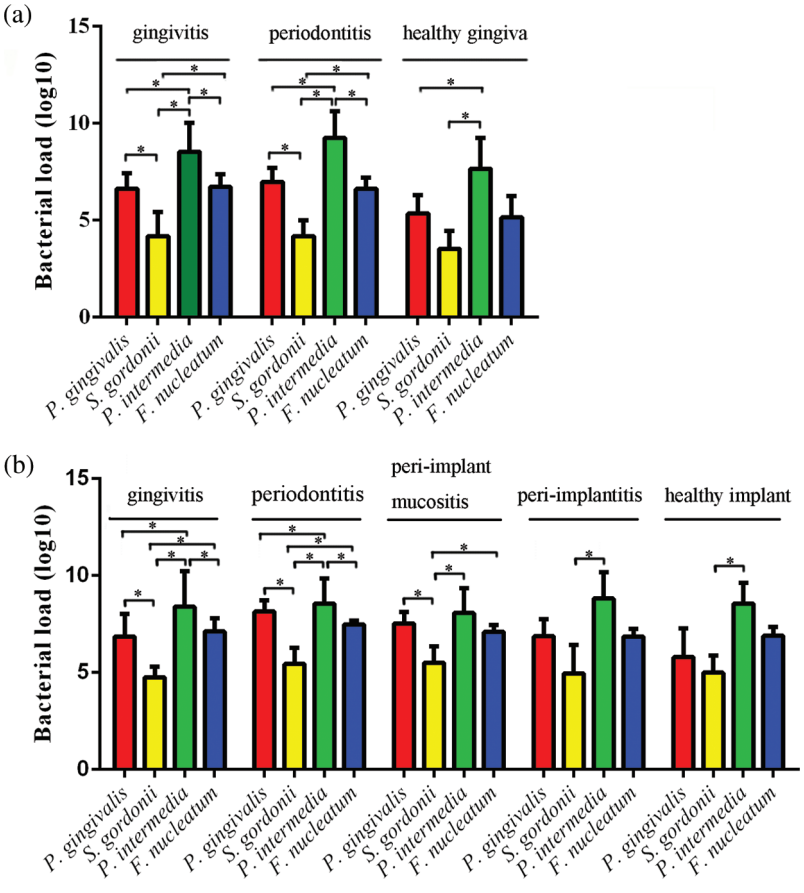

FIGURE 2. Bacterial loads of the four core species in the same clinical sites from patients with periodontitis and peri-implantitis. The results were based on the quantitative real-time polymerase chain reaction. All the dates were conducted in triplicate and tested by the Kruskal-Wallis rank sum test. A significant difference was set at $P < 0.05$ with "*" marked. The colors red, yellow, green, and blue represent *Porphyromonas gingivalis, Streptococcus gordonii, Prevotella intermedia*, and *Fusobacterium nucleatum*. (a) Sites from patients with periodontitis and (b) sites from patients with peri-implantitis.

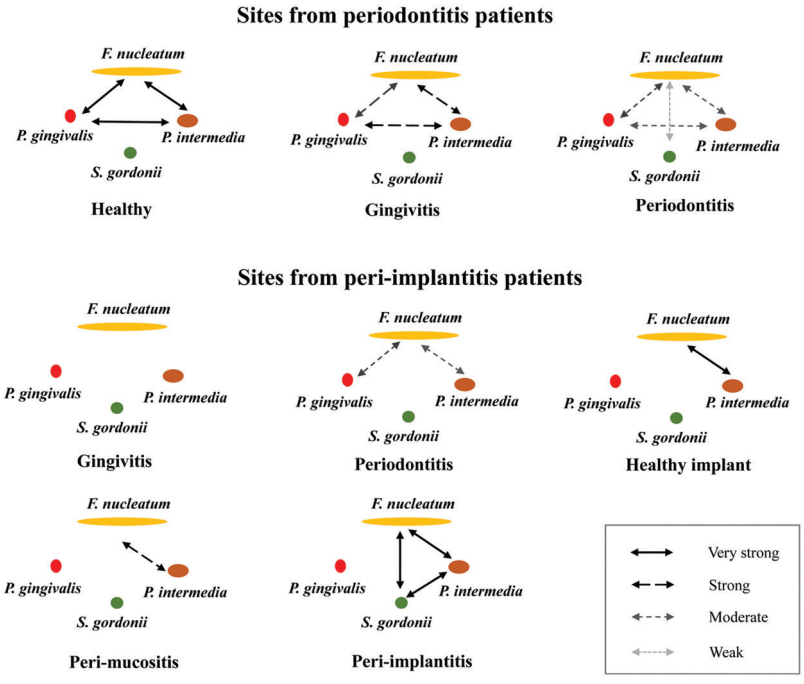

FIGURE 3. Correlations among the four core species at different sites in periodontitis and peri-implantitis samples. Correlations were assessed using Pearson's correlation coefficient and the Kruskal–Wallis rank-sum test based on the results of quantitative real-time polymerase chain reaction. The strength of correlation between two bacteria was classified using *r*-values (0–0.19, very weak; 0.2–0.39, weak; 0.40–0.59, moderate; 0.6–0.79, strong; 0.8–1, very strong). The clinical collected samples were from healthy, gingivitis, and periodontitis sites from periodontitis patients and gingivitis, periodontitis, healthy implant, peri-mucositis, and peri-implantitis sites from peri-implantitis patients. The different types of lines represent different correlations.

Prevotella intermedia at the subgingival plaque in peri-implantitis and periodontitis samples (Supple. Table S1).

Discussion

Many studies have shown that the bacterial community composition and structure in subgingival plaque differ in patients with periodontal disease and healthy individuals (Griffen *et al.*, 2012; Wang *et al.*, 2013; Shi *et al.*, 2015). Certain species have been found to be more frequent and abundant at diseased sites (Gohler *et al.*, 2018; Mullally *et al.*, 2000; Puig-Silla *et al.*, 2017). *Prevotella intermedia* is one of these bacteria. However, in this study, we found no difference in the frequency or abundance of *Prevotella*

intermedia between affected and unaffected sites or between generalized and localized periodontitis. Our results also revealed that under the same oral conditions (generalized and locally recurrent periodontitis), the affected and unaffected sites tended to have similar bacterial loads. However, patients with local periodontitis had significantly lower bacterial loads than the patients with general chronic periodontitis.

As bacterial interactions can influence biofilm formation, metabolic changes, and physiological function, we hypothesized that variation therein would play an important role in oral health status. In our previous study, we concluded that microbial interactions among key species (*F. nucleatum*, *Porphyromonas gingivalis* and *Prevotella intermedia*) have value in managing the subgingival microbial ecosystem through an *in vitro* biofilm model (Zhang *et al.*, 2019), and now we further confirmed it through *in situ* plaque samples. Network analysis has been used to identify interactions among microbiota (i.e., Pearson or Spearman correlation) based on their absolute abundances (Faust and Raes, 2012; Barberan *et al.*, 2012). The positive correlation among *Porphyromonas gingivalis*, *F. nucleatum*, and *Prevotella intermedia* may be due partly to pairwise co-aggregations (Faust and Raes, 2012). Strong (positive or negative) correlations are assumed to have biological, physiological, or ecological significance, possibly resulting from cooperation or competition (Fernandez *et al.*, 2015). The correlation coefficients decreased with the aggravation of periodontal inflammation (healthy–gingivitis–periodontitis). However, only the differences in *Porphyromonas gingivalis*/*F. nucleatum* correlation coefficients between healthy and diseased (gingivitis or periodontitis) sample groups were of statistical significance, suggesting that the interplay of *Porphyromonas gingivalis* and *F. nucleatum* in the subgingival microbiota is affected during the progression of the disease. Although *S. gordonii* can also co-aggregate with *F. nucleatum* and *Porphyromonas gingivalis* (Park *et al.*, 2005), the correlation between *S. gordonii* and *F. nucleatum* was much weaker, and no correlation was detected between *S. gordonii* and *Porphyromonas gingivalis*. These observations suggest that the microbial correlations cannot be explained by mere physicochemical colonization but that cell-cell contact contributes to functional interaction (e.g., metabolic communication and/or genetic exchange) (Guo *et al.*, 2014). The variation in microbial correlation may reflect changes in community function. The network structure was consistent at periodontal healthy and diseased sites, i.e., no correlated pair emerged or was lost, and no positive/negative relation changed to the opposite in association with the periodontal condition.

Implant-supported reconstruction is an ideal treatment for dental defects caused by severe periodontitis. However, significantly increased risks of biological complications around dental implants (i.e., peri-implant mucositis and peri-implantitis) have been reported in patients with histories of periodontitis (Sgolastra *et al.*, 2015; Ting *et al.*, 2018). Peri-implant and periodontal diseases have been reported to have different microbial profiles. *F. nucleatum* and *Prevotella intermedia* have been reported to be core bacterial species associated with periodontitis and peri-implantitis (Colombo and Tanner, 2019). Therefore, we then analyzed microbial relationships in the subgingival microbiota at healthy and diseased peri-implant sites in a cohort of patients with histories of periodontitis. Our results suggest that they are also related closely to periodontitis and peri-implantitis.

The pairwise interplay of *F. nucleatum*/*Prevotella intermedia* and *F. nucleatum*/*Porphyromonas gingivalis* at periodontitis sites were consistent in the two cohorts, whereas no correlation between *Prevotella intermedia* and *Porphyromonas gingivalis* was found in subjects with implants. Patients in the periodontitis group had generalized active periodontitis, whereas those with peri-implantitis were in the maintenance phase after periodontal therapy with localized recurrence. Thus, the subgingival ecosystem may have differed between cohorts. Implants are thought to accumulate less plaque than teeth, whereas the gingivitis microbiome is more diverse than that seen in peri-implant mucositis (Schincaglia *et al.*, 2017). The relative spatial distribution of *Porphyromonas gingivalis* and *Prevotella intermedia* was random (Schillinger *et al.*, 2012), which may explain their dynamic relationship. To determine whether the difference in microbial interplay was affected by variation in bacterial abundance, we compared the absolute abundance of each species between groups and observed no significant change in the abundance of any one of these four species. These results confirm that the changes in interspecies correlations were associated with changes in the microbial ecosystem.

Recent studies have suggested that the microbial relationships shown in correlation interaction networks can be used to determine drivers of disease (Greenblum *et al.*, 2012; Faust and Raes, 2012). To better understand interactions in the human microbiome, most studies have attempted to construct correlation networks with sequencing data. The reads are classified based on similarity to generate profiles (taxonomic or functional) of different microbial samples. This approach is useful for the description of the "social" nature of a microbiome, but the snapshots it provides make little contribution to the description of interactions between community members, and a large amount of metagenomics data renders the recovery of real relationships in the bacterial community difficult (Weiss *et al.*, 2016). Yu *et al.* (2019) constructed a bacterial occurrence network for periodontal and peri-implant microbiota based on 16S metagenomic sequencing. We found little robust agreement between their results and ours, as the correlations in their network were not sufficiently precise. Although we could not obtain a complete picture of the complex interactions that occur in the periodontal and peri-implant microbiota, our findings add to the above-mentioned results by suggesting the biological relevance of interactions between specific core members of the microbiota. Furthermore, the patterns of the strength of interactions between specific bacterial species are associated with different habitats (periodontal and peri-implant, diseased and healthy); even when the same species are present at similar abundance levels in different habitats, their behavior is not necessarily the same. This difference may be due to differences in the local environment, but it could also reflect differences in the presence or absence of other microorganisms.

There were some limitations in our study. Microbiota, as a whole, interact with each other to influence the progress of the oral disease, including periodontitis and peri-implantitis.

We only studied four main pathogenic bacteria; other pathogenic bacteria are also present, for example *Treponema denticola* and *Aggregatibacter actinomycetemcomitans*. Other verified samples should be collected, and the clinically isolated strains should be used for *in vitro* experiments to confirm this conclusion. The influence of bacteria on the progression of periodontal and implant disease in the same patients should be examined to eliminate the differences between individuals.

In summary, the pattern of the correlation network for four core species differed between different periodontal/peri-implant habitats, supporting the hypothesis that the variation in microbiota is relevant to health status. Further studies are required to understand the implications of these interactions and why these differences exist.

Availability of Data: The datasets generated analyzed during the current study are available from the corresponding author on reasonable request.

Author Contribution: Study conceived and design of the experiments: ZYF, CXP; collection of the samples, experimentation and analysis of data: ZQ, ZYF, ZM, LP; manuscript writing and revision: ZQ, ZYF, ZM, CXP. All authors reviewed the results and approved the final draft of the manuscript.

Ethics Approval: The institutional review board of Peking University School and Hospital of Stomatology (Beijing, China) approved the study protocol (No. PKUSSIRB-2012063).

Funding Statement: This study was supported by grants from the Peking University School of Stomatology (PKUSS20170112).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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TABLE S1

The Correlation analysis among the four core species based on metagenomic data in peri-implantitis and periodontitis samples

	Source	Target	r_value	p_value	abs_value	linktype
Periodontitis	Fusobacterium nucleatum	Fusobacterium nucleatum	1	0	1	1
	Prevotella intermedia	Fusobacterium nucleatum	0.725092571	3.01E-16	0.725092571	1
	Porphyromonas gingivalis	Porphyromonas gingivalis	1	0	1	1
	Fusobacterium nucleatum	Prevotella intermedia	0.725092571	1.81E-15	0.725092571	1
	Prevotella intermedia	Prevotella intermedia	1	0	1	1
	Streptococcus gordonii	Streptococcus gordonii	1	0	1	1
Peri-implantitis	Fusobacterium nucleatum	Fusobacterium nucleatum	1	1.09E-173	1	1
	Prevotella intermedia	Fusobacterium nucleatum	0.508695652	0.011135557	0.508695652	1
	Porphyromonas gingivalis	Porphyromonas gingivalis	1	1.09E-173	1	1
	Prevotella intermedia	Prevotella intermedia	1	1.09E-173	1	1
	Streptococcus gordonii	Streptococcus gordonii	1	0	1	1