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ORIGINAL ARTICLE

Mucosal bleeding correlates with submucosal microbial dysbiosis in peri-implant mucositis of patients with periodontitis

Siqi Li[1] | Fei Sun[1] | Yiping Wei[1] | Yong Nie[2] | Xiaolei Wu[2,3,4] | Wenjie Hu[1,5]

[1]Department of Periodontology, National Center of Stomatology, National Clinical Research Center for Oral Diseases, National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing Key Laboratory of Digital Stomatology, Peking University School and Hospital of Stomatology, Beijing, China

[2]College of Engineering, Peking University, Beijing, China

[3]Institute of Ocean Research, Peking University, Beijing, China

[4]Institute of Ecology, Peking University, Beijing, China

[5]NHC Research Center of Engineering and Technology for Computerized Dentistry, Peking University, Beijing, China

Correspondence
Yong Nie and Xiaolei Wu, College of Engineering, Peking University, Beijing 100871, China.
Email: nieyong@pku.edu.cn and xiaolei_wu@pku.edu.cn

Wenjie Hu, Department of Periodontology, National Center of Stomatology, National Clinical Research Center for Oral Diseases, National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing Key Laboratory of Digital Stomatology, Peking University School and Hospital of Stomatology, Beijing 100081, China.
Email: huwenjie@pkuss.bjmu.edu.cn

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Abstract

Objectives: This study aimed to investigate the relationship between microbial communities and the severity of peri-implant mucosal bleeding in peri-implant mucositis.

Materials and Methods: Submucosal plaque samples were collected from 54 implants divided into the healthy implant (HI) group, peri-implant mucositis (PM) group, and peri-implantitis (PI) group. Sequencing of 16S rRNA was performed using the Illumina MiSeq platform. Alpha diversity (i.e., Shannon and Chao index) and beta diversity were used to measure microbial diversity within and between microbial communities, respectively. Differences in microbial taxa between groups were assessed via linear discriminate analysis effect size. Correlation between the modified sulcus bleeding index (mSBI) and microbial dysbiosis index (MDI) was examined using Spearman correlation analysis and linear models.

Results: The submucosal bacterial richness (Chao index) was positively correlated with the mean mSBI in the PM group. As the mean mSBI increased in the PM group, the beta diversity became closer to that of the PI group. In the PM group, the abundances of 47 genera were significantly correlated with the mean mSBI, and the MDI was positively associated with the mean mSBI. Fourteen of the forty-seven genera were discriminative taxa between the HI and PI groups, and the abundances of these biomarkers became closer to those in the PI group in the progression of peri-implant disease.

Conclusions: A higher mSBI value corresponded to a higher risk of microbial dysbiosis in peri-implant mucositis. The biomarkers identified may be useful for monitoring the progression of peri-implant disease.

KEYWORDS
microbial dysbiosis index , microbiome, modified sulcus bleeding index , peri-implant mucositis

Clinical trial registration number: ChiCTR2000031392

1 | INTRODUCTION

Dental implants are widely used owing to their favorable performance in prosthetic rehabilitation (Buser et al., 2017). With the increasing use of implants, peri-implant disease, a biological complication after implant insertion, has received much attention worldwide (Karlsson et al., 2020; Shimchuk et al., 2021; Tsigarida et al., 2020). Peri-implant disease is classified into two clinical conditions: peri-implant mucositis (PM) and peri-implantitis (PI) (Caton et al., 2018). Per–implant mucositis (PM) is a reversible inflammatory condition limited to peri-implant soft tissue, while peri–implantitis (PI) is characterized by mucosal inflammation signs and supporting bone loss (Buser et al., 2017; Caton et al., 2018). Peri-implant disease requires time-consuming and costly treatment; if uncontrolled, it can result in implant loss (Abrahamsson et al., 2017; Marcantonio Junior et al., 2019). As an intermediate stage, PM can revert to healthy status after appropriate treatment, but the untreated PM may progress to PI (Schwarz et al., 2018). Thus, a better understanding of PM may help relieve this burden.

The microbiota of plaque is a well-established contributing factor for peri-implant disease (Carcuac et al., 2013; Caton et al., 2018). In recent years, 16S ribosomal RNA (rRNA) gene sequencing analysis has contributed to understanding the detailed characterization of microbial communities. Numerous studies have been conducted to investigate peri-implant submucosal microbial profiles. Most of these studies focused on the peri-implant microbial community features associated with the healthy implant (HI) and PI conditions (Apatzidou et al., 2017; Gao et al., 2018; Sanz-Martin et al., 2017). Controversies existed in the microbiome of peri-implant mucositis. Polymeri et al. (2021) suggested that the microbial profiles of PM were more similar to those found in HI rather than PI. In contrast, the occurrence of disease is accompanied by obvious changes in the microbial community (Ghensi et al., 2020; Tsigarida et al., 2015). A study showed that the microbiome characteristics were similar in PM and PI (Shi et al., 2022). Thus, further work is needed to explore the structure of the PM-associated microbiome.

Peri-implant mucositis (PM) is mainly caused by the disruption of host–microbial homeostasis at the implant–mucosa interface (Salvi et al., 2012; Zitzmann et al., 2001). Identifying the severity of PM through clinical examination is important to monitor the host inflammatory reaction to microbial infection. Erythema, swelling, bleeding, and/or suppuration are typical clinical manifestations (Caton et al., 2018). Various researchers have sought to determine the correlation between peri-implant mucosal inflammation and the microbiome. Submucosal microbial dysbiosis in PM was not found to be related to peri-implant probing depth (PPD) or bleeding on probing (Shi et al., 2022). PM sites with suppuration had more unbalanced microbial community composition with more pathogenic microorganisms (Wang et al., 2020). Regrettably, studies to date have not established a relationship between the grade of bleeding on probing and the submucosal microbiome in PM.

The present study aimed to investigate the association between microbial community profiles and peri-implant mucosal inflammation, as well as to explore the role of the PM microbiome in the progression of peri-implant disease.

2 | MATERIALS AND METHODS

2.1 | Design and participant selection

This study was conducted as a case–control study. The research adhered to the Declaration of Helsinki and was approved by the Ethics Committee of Peking University School and the Hospital of Stomatology (PKUSSIRB-201946080). Written informed consent was obtained from all participants prior to their participation.

Participants who met the inclusion criteria were recruited at the Department of Periodontology in Peking University School and Hospital of Stomatology from September 2019 to September 2020. The inclusion criteria were defined as follows: (i) age ≥ 18 years, (ii) at least 1 implant, (iii) implants with at least 6 months, and (iv) a history of periodontitis. The exclusion criteria were defined as follows: (i) edentulous patients, (ii) used antibiotics in the 6 months before enrollment, (iii) treatment of peri-implant disease in the preceding 6 months, (iv) pregnancy or lactation, and (v) systemic diseases (cardiovascular disease, kidney disease, uncontrolled diabetes, autoimmune deficiency syndrome, hepatitis, etc.).

Diagnostic criteria were based on the consensus report of the 2017 World Workshop on the classification of periodontal and peri-implant diseases and conditions (Berglundh et al., 2018). If more than one implant was present in a subject, the implant with the most severe condition was included in the study. Based on that criterion, one implant was randomly selected when there were multiple healthy implants in a participant.

2.2 | Sampling and clinical evaluation

To prevent influencing the microbial communities, peri-implant submucosal plaque sampling was conducted prior to the clinical examination. The sampling sites were isolated with cotton rolls and air dried. After removing the supramucosal plaque, six sterile paper points (ISO #35) were gently inserted into the bottom of the peri-implant sulcus to collect plaque from six sites (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual) for 30 s. All paper points were pooled in a sterile Eppendorf tube. The approach of plaque elution was consistent with the method described by Lu et al. (2022). Then, the samples were stored at −80°C for further processing.

The modified sulcus bleeding index (mSBI) (Mombelli et al., 1987), PPD, and suppuration (SUP) were evaluated with a light force (approximately 0.25 N) at six sampling sites for each implant. We used mSBI as a measure of bleeding degree (score 0–3) to reflect the severity of peri-implant mucosal inflammation according to the standard previously described by Mombelli et al. (1987). The plaque index (PLI) was also evaluated. Periapical radiographs acquired with the parallel technique were obtained to assess the peri-implant marginal bone level (MBL).

Clinical examination of each patient was performed independently by a single calibrated examiner. The intra-examiner reproducibility was determined through repeated examinations of 10 implants with a 1-hour interval. The data consistency was evaluated

by the Kappa (κ) value. The κ-value for intra-observer agreement on clinical parameters was 0.92, indicating high agreement.

2.3 | DNA extraction and sequencing

Microbial community genomic DNA was extracted using FastDNA® Spin Kit for Soil (MP Biomedicals) according to the manufacturer's instructions. The extracted DNA was checked by 1% agarose gel electrophoresis, and DNA concentration and purity were determined with a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific). The hypervariable V3-V4 region of the bacterial 16S rRNA gene was amplified with the primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) by an ABI GeneAmp® 9700 PCR thermocycler (ABI). PCR amplification of the 16S rRNA gene was performed as follows: initial denaturation at 95°C for 3 min, followed by 27 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s, with a single extension at 72°C for 10 min, ending with a hold at 10°C. The PCR mixtures containing 4 μL of 5×TransStart FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of forward primer (5 μM), 0.8 μL of reverse primer (5 μM), 0.4 μL of TransStart FastPfu DNA Polymerase, 10 ng of template DNA, and ddH_2O up to 20 μL. PCRs were performed in triplicate. The PCR product was extracted from a 2% agarose gel, purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences) according to the manufacturer's instructions, and quantified using a Quantus™ Fluorometer (Promega).

Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq PE300 platform (Illumina) according to the standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. The raw reads of amplicon sequencing were uploaded to the NCBI Sequence Read Archive (SRA) database (BioProject ID: PRJNA887606).

2.4 | 16S rRNA sequencing data analysis

The raw 16S rRNA gene sequencing reads were demultiplexed and processed using the QIIME2 pipeline (v2019.7) (Bolyen et al., 2019). The primer sequences and barcodes were removed using Cutadapt (Martin, 2011). Sequence denoising and clustering into amplicon sequence variants (ASVs) were conducted using DADA2 (Callahan et al., 2016). Taxonomic classification of the ASVs was achieved using the Silva-138-99-nb classifier (Yilmaz et al., 2014). ASVs classified as mitochondria or chloroplasts were eliminated. Singleton ASVs were also discarded. Alpha diversity and beta diversity were determined using the 'core-metrics-phylogenetic' plugin. All samples were rarefied to an even depth of 21,185 sequences.

2.5 | Statistical analysis

One-way ANOVA, Chi-square analysis, Kruskal–Wallis H test, or Fisher exact test was used as appropriate for the clinical parameter analyses. $p < .05$ was the threshold for statistical significance. The microbial alpha diversity of each sample was measured using the Shannon index and Chao index (Chao, 1984; Shannon, 1997). The Shannon index was calculated to evaluate microbial community richness and evenness. The Chao index estimates microbial richness. Student's *t*-test was used to compare significant differences between different groups. The beta diversity was estimated based on Bray–Curtis distance among samples. Statistical significance between groups was confirmed using permutational multivariate analysis of variance (PERMANOVA); p values <.05 were significantly different. Linear discriminate analysis effect size (LEfSe) analysis was used to identify statistically different biomarkers between groups (Segata et al., 2011). A size-effect threshold of 3.0 on the logarithmic linear discriminant analysis (LDA) score was used to identify discriminating taxa from the phylum to the genus level. We calculated the microbial relative abundance, that is, the proportion of a specific taxon's abundance over the total taxon abundance at the same taxonomic level. Associations between the mean mSBI and abundances of discriminating taxa were calculated using Spearman correlation analysis. The microbial dysbiosis index (MDI) was calculated to assess microbiome dysbiosis (Gevers et al., 2014). Microbial abundances were presented in the form of a heatmap. Analyses were performed using IBM SPSS Statistics (version 23.0) and R 4.2.1.

3 | RESULTS

3.1 | Characteristics of the participants and implants

A total of 54 implants from 54 participants were included in this study. The demographic and clinical characteristics of these samples are presented in Table 1. Pairwise comparisons between groups revealed that there were no significant differences in gender, age, smoking status, location of sampled site, and suppuration. The HI group showed a lower PPD than the PM and PI groups ($p < .05$). The PM and PI groups showed significantly higher mSBI ($p < .05$) in comparison with the HI group, and there was no significant difference in mSBI between PM and PI ($p > .05$). The PI group showed higher PLI than the HI group ($p < .05$). The PI group showed significantly higher MBL in comparison with the HI and PM groups ($p < .05$).

3.2 | Sequencing results

Sequencing yielded 2,046,118 sequences from peri-implant submucosal plaque. To correct for variations in sequencing depth, all samples were randomly sampled to 21,185 sequences. Each sample's rarefaction curve (Figure S1) was examined, and it was discovered to approach saturation, proving that the sequencing depth was sufficient. We found a total of 3435 bacterial ASVs taxonomically classified into 25 phyla, 58 classes, 134 orders, 219 families, and 452 genera.

TABLE 1 Demographic and clinical characteristics of the 54 individuals.

	HI ($n=10$)	PM ($n=29$)	PI ($n=15$)	p-value
Gender: female/male	6/4	15/14	6/9	.596
Age, years [mean ± SD]	49.20 ± 12.72	53.79 ± 10.67	52.60 ± 13.56	.469
Smoking status: Nonsmoker/smoker	10/0	27/2	11/4	.074
Location of sampled site				
Maxillary/Mandibular	5/5	19/10	8/7	.593
Anterior/Posterior	0/10	2/27	3/12	.296
PPD, mm [mean ± SD]	2.43 ± 0.35 [a]	4.00 ± 1.25 [b]	7.39 ± 1.56 [c]	<.001
mSBI [mean ± SD]	0.00 ± 0.00 [a]	1.71 ± 1.10 [b]	2.67 ± 0.49 [b]	<.001
PLI [mean ± SD]	0.15 ± 0.24 [a]	0.72 ± 0.69 [ab]	1.27 ± 1.13 [b]	.014
SUP: Non-SUP/SUP	10/0	26/3	10/5	.047
MBL, mm [mean ± SD]	1.02 ± 0.87 [a]	1.03 ± 0.72 [a]	5.29 ± 2.38 [b]	<.001

Abbreviations: HI, healthy implant group; MBL, marginal bone level; mSBI, modified sulcus bleeding index; PI, peri-implantitis group; PLI, plaque index; PM, peri-implant mucositis group; PPD, peri-implant probing depth; SUP, suppuration.

Note: Means with no letter or with the same letter indicate no significant differences ($p > .05$).

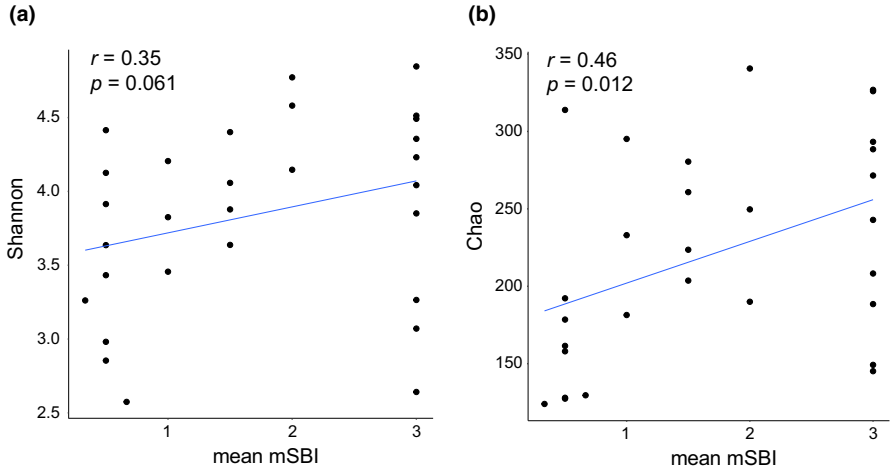

FIGURE 1 Alpha diversity of submucosal microbiome in PM samples with different degrees of mucosal inflammation. (a) Correlation plots for the mean mSBI with the Shannon index. (b) Correlation plots for the mean mSBI with the Chao index.

3.3 | Correlation of bleeding severity and submucosal microbiome diversity in peri-implant mucositis

There was no significant difference on the alpha diversity of the microbial community among the groups, as indicated by the Shannon and Chao indices (Table S1). To better understand PM, the mean mSBI was used to assess the overall inflammation of peri–implant soft tissue. Notably, Figure 1 showed the Chao index had a significant positive correlation with the mean mSBI ($p = .012$), and the Shannon index was not found to be correlated with the mean mSBI ($p = .061$).

To assess the variation in microbial profiles between peri-implant health and peri-implant disease, we calculated the beta diversity. Principal coordinate analysis (PCoA) showed significant differences ($p = .012$) among the three conditions, as shown in Figure 2a. Interestingly, samples of the PM group were scattered between those of the HI and PI groups. Pairwise PERMANOVA results showed the beta diversity of the PI group was higher than that of the HI group ($p = .006$) in Figure 2b. When compared to PM, neither condition showed significant differences ($p > .05$), but as the mean mSBI value increased, the microbial profile of PM transformed from being HI-like to PI-like.

3.4 | Taxa related to disease severity and dysbiosis evaluation in peri-implant mucositis

The bar plot (Figure 3a) shows the distribution of the top 10 phyla in the 54 samples. The peri-implant submucosal bacterial communities were dominated by representatives of *Firmicutes* (32.53% in HI, 34.68% in PM, and 32.24% in PI), *Proteobacteria* (32.11% in HI, 23.79% in PM, and16.34% in PI), *Actinobacteriota* (14.46% in HI, 10.58% in PM, and 8.66% in PI), *Fusobacteriota* (9.74% in HI, 7.29% in PM, and 9.85% in PI), and *Bacteroidota* (6.76% in HI, 14.94% in PM, and 20.30% in PI). At the phylum level, *Desulfobacterota*, *Bacteroidota*, *Spirochaetota*, and *Synergistota* showed higher abundance in the PI group compared to the HI group, while *Proteobacteria* showed lower

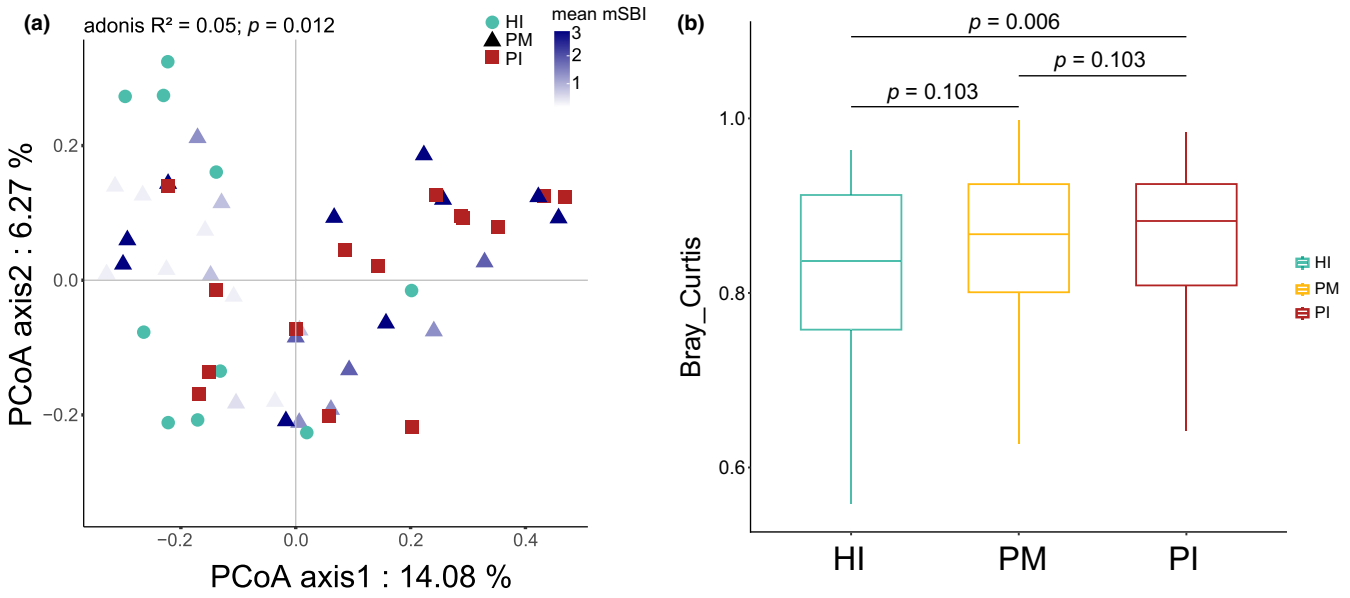

FIGURE 2 Beta diversity of microbial communities in the three conditions. (a) Principal coordinate analysis (PCoA) of the microbiome based on the Bray–Curtis distances. The colors of the bars represent the mean mSBI score: the bluer the color is, the larger the mean mSBI is. (b) Boxplots comparing Bray-Curtis distances between various groups. Significance was assessed using permutational multivariate analysis of variance (PERMANOVA).

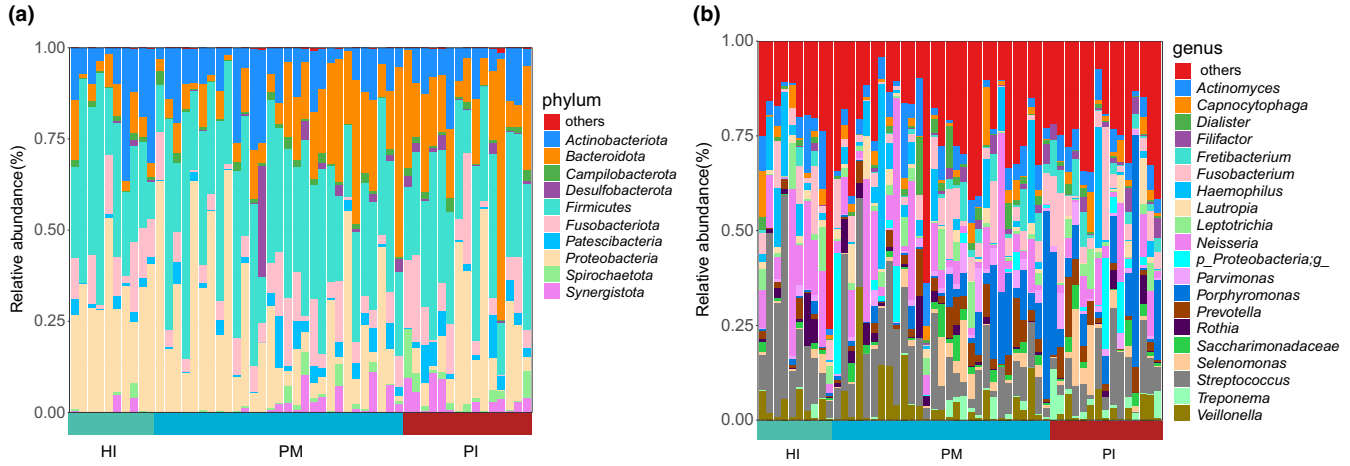

FIGURE 3 Taxonomic composition signature of microbial communities under the three conditions. (a) Distribution of the top 10 phyla in each sample. (b) Distribution of the top 20 genera in each sample. *Note*: PM samples are arranged by their mean mSBI.

abundance in the PI group (Figure 4a). The relative abundances of *Patescibacteria*, *Desulfobacterota*, *Spirochaetota*, *Synergistota*, and *Bacteroidota* were significantly positively correlated with the mean mSBI in PM, while the relative abundance of *Proteobacteria* was significantly negatively correlated with the mean mSBI (Figure 4b).

At the genus level, the 20 most abundant genera were identified, accounting for 76.18% in HI, 75.97% in PM, and 75.08% in PI (Figure 3b). We investigated 23 significantly different genera between the HI and PI groups (Figure 4a). *Filifactor*, *Fretibacterium*, *Haemophilus*, *Neisseria*, *Parvimonas*, *Porphyromonas*, *Rothia*, and *Treponema* were the top 20 most abundant genera (Figure 3b). We further explored the relationship between the relative abundances of genera and the mean mSBI in the PM samples. We found that the relative abundances of 47 genera were significantly correlated with the mean mSBI (Figure 4c). Based on these genera, the MDI in PM was calculated to evaluate microbial dysbiosis. The formula was established as follows:

$$\mathrm{MDI} = \log\left(\frac{[\text{total abundance of taxa positively associated with mean mSBI}]}{[\text{total abundance of taxa negatively associated with mean mSBI}]}\right)$$

The results showed that the MDI was positively correlated with the mean mSBI in the PM group (Figure 5).

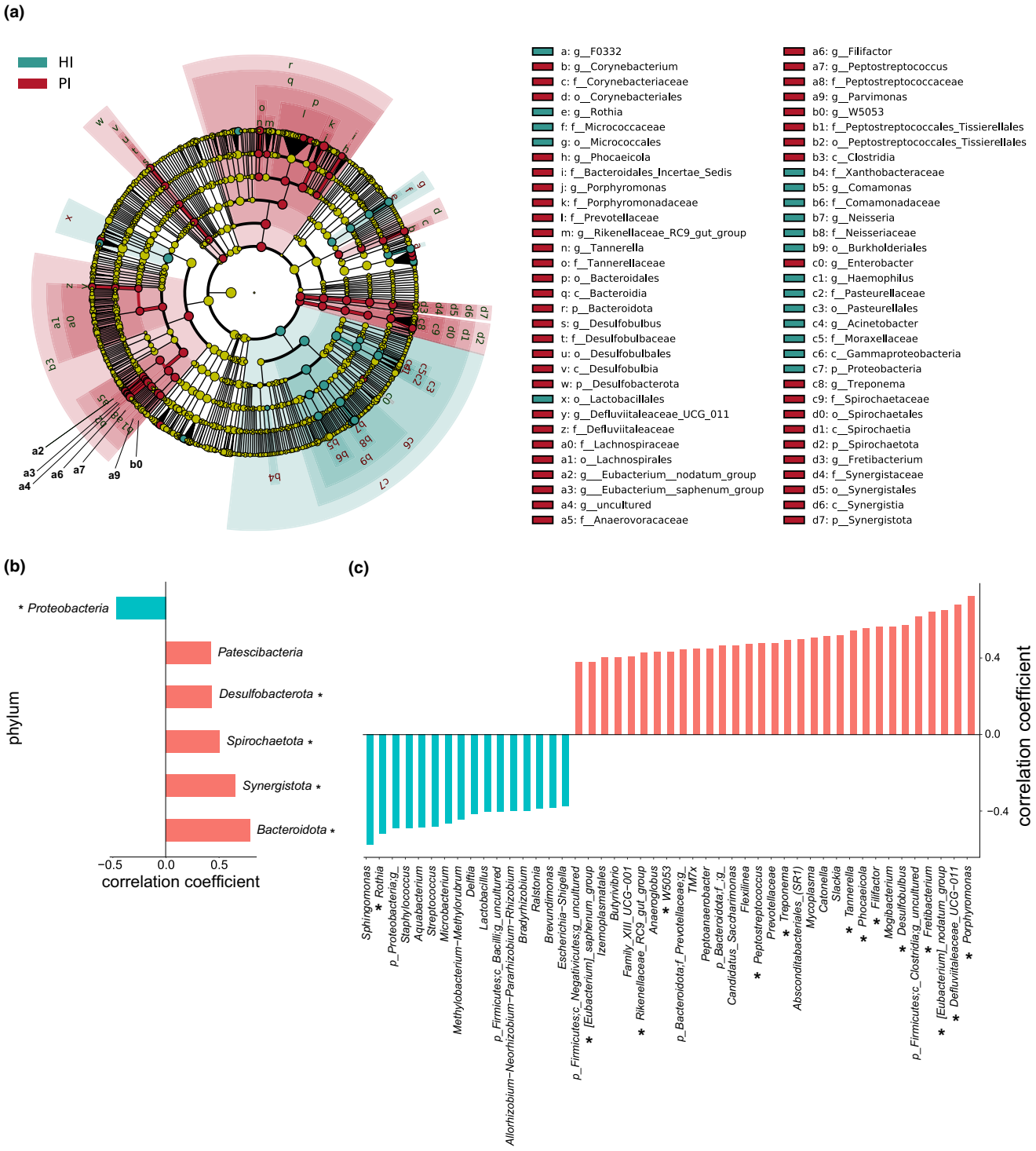

FIGURE 4 Biomarkers in the three peri-implant conditions. (a) LEfSe analysis between the HI and PI groups. The cladogram plots show differential taxa at different levels of taxonomic classification (phylum, class, order, family, and genus). Only taxa with LDA values ≥3.0 are shown. (b) Relationship between the relative abundances of microbial community composition at the phylum level and mean mSBI in PM based on Spearman correlation analysis ($p < .05$). Differentially abundant phyla in the HI and PI groups are marked with asterisks. (c) Relationship between the relative abundances of microbial community composition at the genus level and mean mSBI in PM based on Spearman correlation analysis ($p < .05$). Differentially abundant genera in the HI and PI groups are marked with asterisks. The red and green colors indicate positive and negative correlations, respectively, and the heights of the columns represent Spearman's correlation coefficients.

3.5 | Changes in biomarkers during peri-implant disease progression

Of the 47 genera observed in Figure 4c, 14 genera were the discriminative taxa between HI and PI, as indicated by asterisks. The relative abundances of *Peptostreptococcus*, *W5053*, *[Eubacterium]_saphenum_group*, *Rikenellaceae_RC9_gut_group*, *Treponema*, *Tannerella*, *Filifactor*, *Phocaeicola*, *Desulfobulbus*, *Fretibacterium*, *[Eubacterium]_nodatum_group*, *Defluviitaleaceae_UCG−011*, and *Porphyromonas* were positively correlated with the mean mSBI, while that of *Rothia* was negatively correlated with the mean mSBI (Figure 6).

To gain further insight into the characteristics of microbial changes in the progression of peri-implant disease, a heatmap including the above 14 differential genera in the three groups was created in Figure 7. The abundances of these genera in the PM samples with less severe mucosal inflammation were similar to those in the HI samples. PM samples with more severe mucosal inflammation showed microbial abundances were similar to those of the PI samples.

4 | DISCUSSION

To our knowledge, this is the first study to explore the relationship between the mucosal bleeding severity and the submucosal microbiome in peri-implant mucositis. The submucosal microbiome of PM exhibited different microbial structures based on the degree of mucosal inflammation. Mucosal inflammation was associated with microbial dysbiosis in PM. These findings emphasize the critical role of peri-implant mucositis in the progression of peri-implant disease.

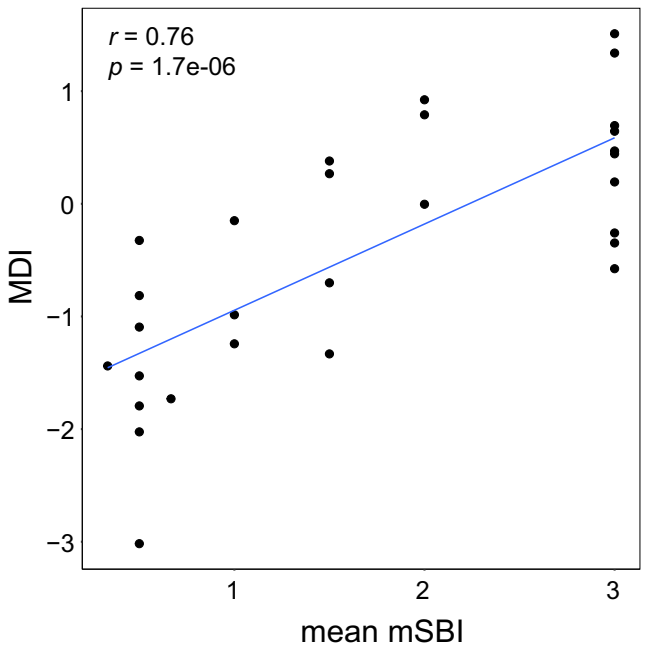

FIGURE 5 Correlation between the microbial dysbiosis index (MDI) of submucosal microbiome and the mean mSBI in the PM group.

The key pathogenic microbes in PM may be beneficial for our understanding of the disease progression.

The presence of soft tissue inflammation is the typical clinical indication of peri-implant mucositis. The microbial community structure of sites with suppuration was different from that of sites without suppuration (Wang et al., 2020). A strong relationship was observed between bleeding on probing and the subgingival microbiome, but it was not observed in the peri-implant submucosal microbiome (Camelo-Castillo et al., 2015; Polymeri et al., 2021). The sensitivity of bleeding on probing is lower compared to a graded bleeding index such as the mSBI (Newbrun, 1996). In the current study, we found that the richness of the microbial profile increased with the mean mSBI in PM. This correlation could be attributed to the weak attachment between the mucosa and the implant (Atsuta et al., 2016). Disruption of the soft tissue seal around dental implants might facilitate bacterial invasion.

In agreement with previous studies (Apatzidou et al., 2017; Gao et al., 2018; Ghensi et al., 2020; Sanz-Martin et al., 2017), the submucosal microbial communities between HI and PI sites were significantly different in our study. The beta diversity in the PI group was higher than the HI group in our study identifying more variability in the microbial communities of PI group. However, this result is contrary to previous studies (Ghensi et al., 2020; Zheng et al., 2015), which may be due to the different metagenomic sequencing depths (16S rRNA vs. shotgun sequencing) or to the difference in the sampling procedure. Previous studies have in fact sampled at the deepest probing site of each implant (Ghensi et al., 2020; Zheng et al., 2015). While we collected the submucosal plaque from six sites around each implant, this method may allow a different overview of the peri-implant microbial profile. We deduced the occurrence of these results resulted from high amplitude of variation in the peri-implant microenvironment. The products related to the inflammatory response in peri-implant disease could be used as nutrients for microbes, which may induce a greater number of ecological niches.

The signature of the submucosal microbiome in peri-implant mucositis is still debatable. The microbiome structure of PM was similar to that of PI (Shi et al., 2022), while Polymeri et al. (2021) observed that the microbiome structure of PM was similar to that of HI. A wide distribution of PM samples was observed in our study. We found that PM samples with lighter bleeding showed a microbial community structure similar to the HI group, while the microbiome of PM samples with severe bleeding was similar to that in the PI samples. This highlights the importance of conducting comprehensive sampling at PM sites with varying degrees of severity to study the microbiome characteristics of PM. Additionally, it is a reminder that it is better to separate PM samples in future analysis, especially when investigating the microbiome.

Different PPD, disease status, dentition status, and implant location were associated with distinct microbial profiles (Polymeri et al., 2021; Shi et al., 2018). Research (Kröger et al., 2018) showed that deeper peri-implant pockets showed a more dysfunctional microbial pattern in peri-implantitis. However, Shi et al. (2022) found that only marginal bone loss was correlated with microbial dysbiosis

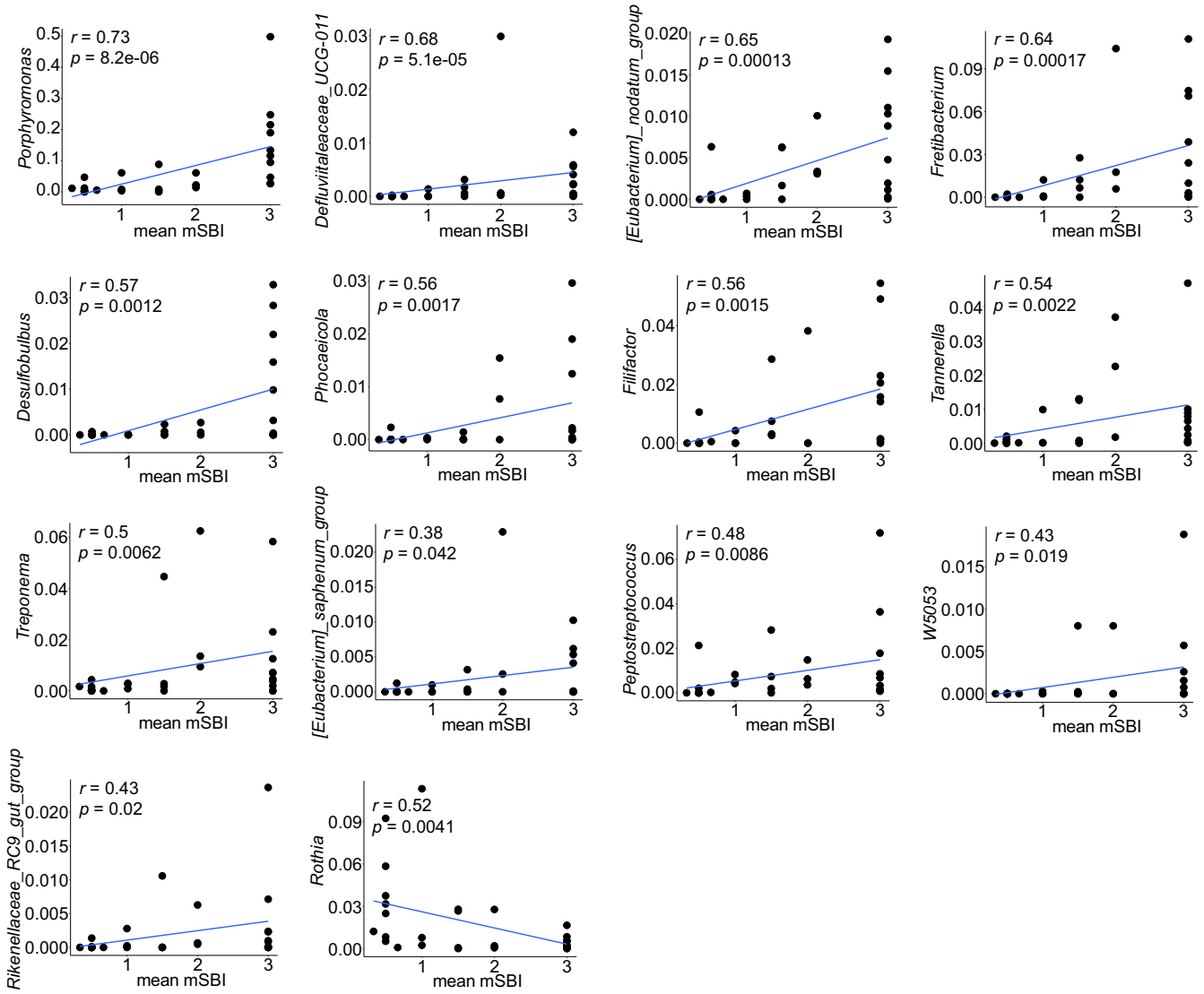

FIGURE 6 Correlation analysis of the mean mSBI in PM and differentially abundant 14 genera, which were observed comparing the HI group with the PI group ($p < .05$).

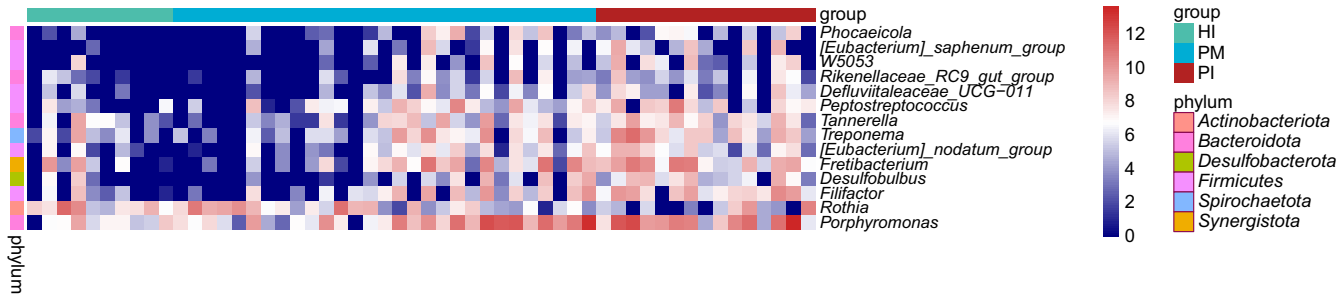

FIGURE 7 Transition pattern of biomarkers from healthy implants to peri-implant disease. The heatmap shows the abundances of bacterial taxa (log2-transformed); red indicates high abundance, and blue indicates low abundance.

in peri-implant disease. We observed a correlation between mucosal inflammation and microbiome imbalance in PM for the first time.

Through investigating the microbial composition under peri-implant health and disease, we found that *Firmicutes*, *Proteobacteria*, *Actinobacteriota*, *Fusobacteriota*, and *Bacteroidota* were generally the most abundant phyla, as previously stated (Al-Ahmad et al., 2018; Sousa et al., 2017; Wang et al., 2020). Previous research has shown distinct biomarkers at the genus level under different peri-implant conditions (Belibasakis & Manoil, 2020; de Melo et al., 2020; Kumar et al., 2012; Shi et al., 2022). In our study, LEfSe was used to identify

discriminative phyla and genera between HI and PI, and most of the differentially abundant genera were significantly correlated with the mean mSBI in PM, which might indicate that this change during PM contribute to microbial dysbiosis.

Numerous taxa were associated with mucosal bleeding. The enrichment of *Porphyromonas*, *Defluviitaleaceae_UCG−011*, *[Eubacterium]_nodatum_group*, *Fretibacterium*, *Desulfobulbus*, *Phocaeicola*, *Filifactor*, *Tannerella*, *Treponema*, *Peptostreptococcus*, *W5053*, *Rikenellaceae_RC9_gut_group*, and *[Eubacterium]_saphenum_group* was correlated with increasing mean mSBI. These genera could serve as indicators of disease progression. Some of these genera have been regarded as pathogenic peri-implant microbes, such as *Filifactor* (Barbagallo et al., 2021); *Treponema*, *Fretibacterium*, and *Desulfobulbus* (Korsch et al., 2021; Polymeri et al., 2021); *Tannerella* (Wang et al., 2020); *Porphyromonas* (Yu et al., 2019). Additionally, we observed several genera that were not reported in peri–implant disease previously. *Defluviitaleaceae_UCG−011*was related to salivary microbiota dysbiosis, which was a potential biological predictor of high risk for rheumatoid arthritis (Tong et al., 2020). *Phocaeicola* existed predominantly in the saliva of periodontitis subjects (Lundmark et al., 2019). *W5053* and *Rikenellaceae_RC9_gut_group* were associated with mental illness and Parkinson's disease (Arikan et al., 2022; Yan et al., 2021). Moreover, abundance alteration could happen to part of the 14 genera screened after the treatment of peri-implant disease. The relative abundance of *Tannerella* and *Porphyromonas* decreased after nonsurgical treatment for peri-implantitis, whereas the relative abundance of *Rothia* increased (Shiba et al., 2021). A study found *Porphyromonas* and *Treponema* could be influenced by peri-implantitis treatment (Nie et al., 2020). Bacterial species belonging to the genera *Porphyromonas*, *Tannerella*, *Peptostreptococcus*, *Filifactor*, *and Desulfobulbus*, such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Peptostreptococcaceae XIG-6 nodatum*, *Filifactor alocis*, and *Desulfobulbus* sp. *HMT 041*, decreased after mechanical debridement treatment (Sun et al., 2022).

Sequencing of 16S rRNA amplicons has been widely used, and most studies adopt operational taxonomic unit (OTU) clustering for data analysis. ASVs make marker gene sequencing more precise, reusable, and reproducible than OTUs (Callahan et al., 2017). Analyzing data in this way ensures the acquisition of more high-quality, reliable results.

However, our results must be considered in light of several limitations. A limitation of this study is the heterogeneity in inclusion criteria. This study enrolled subjects with a history of periodontitis, six of them were smokers. These may lead to potential effects on the peri-implant submucosal microbiome. Future studies with adequate control of confounding factors are required to confirm our findings. The sampling method in the study was based on the use of sterile paper points. Compared to using sterile curettes, this approach may lead to a higher risk of contamination with exogenous DNA (van der Horst et al., 2013). It is advisable to sample with sterile curettes for further research. Moreover, our study has a sample size limitation, and the proportions of many clinical features are relatively low. So, we could not explore the relationship between the mucosal bleeding and the microbiome in PI and the contribution of multiple clinical parameters or factors on peri-implant submucosal microbiome precisely. Future studies with larger sample sizes are needed to explore the features of peri-implant conditions from a microbial perspective. More research on host–microbe interactions needs to be undertaken to gain further insights into peri-implant disease pathogenesis mechanisms.

5 | CONCLUSION

This study contributes to our understanding of the microbiome in peri-implant mucositis and its role in the progression of peri-implant disease. The mSBI is a clinical risk indicator for the change in the submucosal microbial community and microbial dysbiosis in peri-implant mucositis. The 14 biomarkers found in this study may be beneficial for the monitoring and treatment of peri-implant disease.

AUTHOR CONTRIBUTIONS

Siqi Li, Yong Nie, Xiaolei Wu, and Wenjie Hu designed the study. Siqi Li, Fei Sun, and Wenjie Hu recruited patients and collected oral samples. Siqi Li performed the analysis and wrote the original draft. Siqi Li and Yiping Wei interpreted the data. Yong Nie, Xiaolei Wu, and Wenjie Hu supervised the whole project. All authors reviewed and approved the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in National Center for Biotechnology Information at https://www.ncbi.nlm.nih.gov/, reference number PRJNA887606.

ETHICS STATEMENT

This research was conducted in accordance with the ethical standards detailed in the Declaration of Helsinki.

PATIENT CONSENT

The authors' institutional ethics committee has approved this study and all patients have provided written informed consent.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

No material has been reproduced from other sources.

ORCID

Siqi Li https://orcid.org/0000-0002-0048-0820

Fei Sun https://orcid.org/0000-0002-1343-8808

Yiping Wei https://orcid.org/0000-0002-8517-3757

REFERENCES

Abrahamsson, K. H., Wennström, J. L., Berglundh, T., & Abrahamsson, I. (2017). Altered expectations on dental implant therapy; views of patients referred for treatment of peri-implantitis. *Clinical Oral Implants Research*, *28*(4), 437–442. https://doi.org/10.1111/clr.12817

Al-Ahmad, A., Muzafferiy, F., Anderson, A. C., Wolber, J. P., Ratka-Kruger, P., Fretwurst, T., Nelson, K., Vach, K., & Hellwig, E. (2018). Shift of microbial composition of peri-implantitis-associated oral biofilm as revealed by 16S rRNA gene cloning. *Journal of Medical Microbiology*, *67*(3), 332–340. https://doi.org/10.1099/jmm.0.000682

Apatzidou, D., Lappin, D. F., Hamilton, G., Papadopoulos, C. A., Konstantinidis, A., & Riggio, M. P. (2017). Microbiome of peri-implantitis affected and healthy dental sites in patients with a history of chronic periodontitis. *Archives of Oral Biology*, *83*, 145–152. https://doi.org/10.1016/j.archoralbio.2017.07.007

Arikan, M., Yildiz, Z., Demir, T. K., Yilmaz, N. H., Sen, A., Hanoglu, L., & Yildirim, S. (2022). Axillary microbiota is associated with cognitive impairment in Parkinson's disease patients. *Microbiology Spectrum*, *10*(1), e0235821. https://doi.org/10.1128/spectrum.02358-21

Atsuta, I., Ayukawa, Y., Kondo, R., Oshiro, W., Matsuura, Y., Furuhashi, A., Tsukiyama, Y., & Koyano, K. (2016). Soft tissue sealing around dental implants based on histological interpretation. *Journal of Prosthodontic Research*, *60*(1), 3–11. https://doi.org/10.1016/j.jpor.2015.07.001

Barbagallo, G., Santagati, M., Guni, A., Torrisi, P., Spitale, A., Stefani, S., Ferlito, S., & Nibali, L. (2021). Microbiome differences in periodontal, peri-implant, and healthy sites: A cross-sectional pilot study. *Clinical Oral Investigations*, *26*(3), 2771–2781. https://doi.org/10.1007/s00784-021-04253-4

Belibasakis, G. N., & Manoil, D. (2020). Microbial community-driven etiopathogenesis of peri-implantitis. *Journal of Dental Research*, *100*(1), 21–28. https://doi.org/10.1177/0022034520949851

Berglundh, T., Armitage, G., Araujo, M. G., Avila-Ortiz, G., Blanco, J., Camargo, P. M., Chen, S., Cochran, D., Derks, J., Figuero, E., Hämmerle, C. H. F., Heitz-Mayfield, L. J. A., Huynh-Ba, G., Iacono, V., Koo, K. T., Lambert, F., McCauley, L., Quirynen, M., Renvert, S., … Zitzmann, N. (2018). Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. *Journal of Periodontology*, *89*(Suppl 1), S313–S318. https://doi.org/10.1002/jper.17-0739

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9

Buser, D., Sennerby, L., & De Bruyn, H. (2017). Modern implant dentistry based on osseointegration: 50 years of progress, current trends and open questions. *Periodontology 2000*, *73*(1), 7–21. https://doi.org/10.1111/prd.12185

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal*, *11*(12), 2639–2643. https://doi.org/10.1038/ismej.2017.119

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Camelo-Castillo, A., Novoa, L., Balsa-Castro, C., Blanco, J., Mira, A., & Tomás, I. (2015). Relationship between periodontitis-associated subgingival microbiota and clinical inflammation by 16S pyrosequencing. *Journal of Clinical Periodontology*, *42*(12), 1074–1082. https://doi.org/10.1111/jcpe.12470

Carcuac, O., Abrahamsson, I., Albouy, J. P., Linder, E., Larsson, L., & Berglundh, T. (2013). Experimental periodontitis and peri-implantitis in dogs. *Clinical Oral Implants Research*, *24*(4), 363–371. https://doi.org/10.1111/clr.12067

Caton, J. G., Armitage, G., Berglundh, T., Chapple, I. L. C., Jepsen, S., Kornman, K. S., Mealey, B. L., Papapanou, P. N., Sanz, M., & Tonetti, M. S. (2018). A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. *Journal of Clinical Periodontology*, *45*, S1–S8. https://doi.org/10.1111/jcpe.12935

Chao, A. (1984). Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics*, *11*, 265–270.

de Melo, F., Milanesi, F. C., Angst, P. D. M., & Oppermann, R. V. (2020). A systematic review of the microbiota composition in various peri-implant conditions: Data from 16S rRNA gene sequencing. *Archives of Oral Biology*, *117*, 104776. https://doi.org/10.1016/j.archoralbio.2020.104776

Gao, X., Zhou, J., Sun, X., Li, X., & Zhou, Y. (2018). Diversity analysis of subgingival microbial bacteria in peri-implantitis in Uygur population. *Medicine (Baltimore)*, *97*(5), e9774. https://doi.org/10.1097/md.0000000000009774

Gevers, D., Kugathasan, S., Denson, L. A., Vazquez-Baeza, Y., Van Treuren, W., Ren, B., Schwager, E., Knights, D., Song, S. J., Yassour, M., Morgan, X. C., Kostic, A. D., Luo, C., González, A., McDonald, D., Haberman, Y., Walters, T., Baker, S., Rosh, J., … Xavier, R. J. (2014). The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host & Microbe*, *15*(3), 382–392. https://doi.org/10.1016/j.chom.2014.02.005

Ghensi, P., Manghi, P., Zolfo, M., Armanini, F., Pasolli, E., Bolzan, M., Bertelle, A., Dell'Acqua, F., Dellasega, E., Waldner, R., Tessarolo, F., Tomasi, C., & Segata, N. (2020). Strong oral plaque microbiome signatures for dental implant diseases identified by strain-resolution metagenomics. *NPJ Biofilms Microbiomes*, *6*(1), 47. https://doi.org/10.1038/s41522-020-00155-7

Karlsson, K., Derks, J., Wennström, J. L., Petzold, M., & Berglundh, T. (2020). Occurrence and clustering of complications in implant dentistry. *Clinical Oral Implants Research*, *31*(10), 1002–1009. https://doi.org/10.1111/clr.13647

Korsch, M., Marten, S. M., Stoll, D., Prechtl, C., & Dötsch, A. (2021). Microbiological findings in early and late implant loss: An observational clinical case-controlled study. *BMC Oral Health*, *21*(1), 112. https://doi.org/10.1186/s12903-021-01439-w

Kröger, A., Hülsmann, C., Fickl, S., Spinell, T., Hüttig, F., Kaufmann, F., Heimbach, A., Hoffmann, P., Enkling, N., Renvert, S., Schwarz, F., Demmer, R. T., Papapanou, P. N., Jepsen, S., & Kebschull, M. (2018). The severity of human peri-implantitis lesions correlates with the level of submucosal microbial dysbiosis. *Journal of Clinical Periodontology*, *45*(12), 1498–1509. https://doi.org/10.1111/jcpe.13023

Kumar, P. S., Mason, M. R., Brooker, M. R., & O'Brien, K. (2012). Pyrosequencing reveals unique microbial signatures associated with healthy and failing dental implants. *Journal of Clinical Periodontology*, *39*(5), 425–433. https://doi.org/10.1111/j.1600-051X.2012.01856.x

Lu, H., Zou, P., Zhang, Y., Zhang, Q., Chen, Z., & Chen, F. (2022). The sampling strategy of oral microbiome. *iMeta*, *1*(2), e23. https://doi.org/10.1002/imt2.23

Lundmark, A., Hu, Y. O. O., Huss, M., Johannsen, G., Andersson, A. F., & Yucel-Lindberg, T. (2019). Identification of salivary microbiota and its association with host inflammatory mediators in periodontitis.

Frontiers in Cellular and Infection Microbiology, *9*, 216. https://doi.org/10.3389/fcimb.2019.00216

Marcantonio Junior, E., Romito, G. A., & Shibli, J. A. (2019). Peri-implantitis as a "burden" disease. *Brazilian Oral Research*, *33*(suppl 1), e087. https://doi.org/10.1590/1807-3107bor-2019.vol33.0087

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10–12. https://doi.org/10.14806/ej.17.1.200

Mombelli, A., Van Oosten, M. A., Schurch, E., & Land, N. P. (1987). The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiology and Immunology*, *2*(4), 145–151. https://doi.org/10.1111/j.1399-302x.1987.tb00298.x

Newbrun, E. (1996). Indices to measure gingival bleeding. *Journal of Periodontology*, *67*(6), 555–561. https://doi.org/10.1902/jop.1996.67.6.555

Nie, J., Zhang, Q., Zheng, H., Xu, L. X., Wang, X. Y., & Chen, F. (2020). Pyrosequencing of the subgingival microbiome in peri-implantitis after non-surgical mechanical debridement therapy. *Journal of Periodontal Research*, *55*(2), 238–246. https://doi.org/10.1111/jre.12708

Polymeri, A., van der Horst, J., Buijs, M. J., Zaura, E., Wismeijer, D., Crielaard, W., Loos, B. G., Laine, M. L., & Brandt, B. W. (2021). Submucosal microbiome of peri-implant sites: A cross-sectional study. *Journal of Clinical Periodontology*, *48*(9), 1228–1239. https://doi.org/10.1111/jcpe.13502

Salvi, G. E., Aglietta, M., Eick, S., Sculean, A., Lang, N. P., & Ramseier, C. A. (2012). Reversibility of experimental peri-implant mucositis compared with experimental gingivitis in humans. *Clinical Oral Implants Research*, *23*(2), 182–190. https://doi.org/10.1111/j.1600-0501.2011.02220.x

Sanz-Martin, I., Doolittle-Hall, J., Teles, R. P., Patel, M., Belibasakis, G. N., Hämmerle, C. H. F., Jung, R. E., & Teles, F. R. F. (2017). Exploring the microbiome of healthy and diseased peri-implant sites using Illumina sequencing. *Journal of Clinical Periodontology*, *44*(12), 1274–1284. https://doi.org/10.1111/jcpe.12788

Schwarz, F., Derks, J., Monje, A., & Wang, H. L. (2018). Peri-implantitis. *Journal of Clinical Periodontology*, *45*(Suppl 20), S246–S266. https://doi.org/10.1111/jcpe.12954

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*, *12*(6), R60. https://doi.org/10.1186/gb-2011-12-6-r60

Shannon, C. E. (1997). The mathematical theory of communication. 1963. *MD Computing*, *14*(4), 306–317.

Shi, M., Wei, Y., Hu, W., Nie, Y., Wu, X., & Lu, R. (2018). The subgingival microbiome of periodontal pockets with different probing depths in chronic and aggressive periodontitis: A pilot study. *Frontiers in Cellular and Infection Microbiology*, *8*, 124. https://doi.org/10.3389/fcimb.2018.00124

Shi, Y., Tong, Z., Zhang, Y., Si, M., & He, F. (2022). Microbial profiles of peri-implant mucositis and peri-implantitis: Submucosal microbial dysbiosis correlates with disease severity. *Clinical Oral Implants Research*, *33*(2), 172–183. https://doi.org/10.1111/clr.13880

Shiba, T., Watanabe, T., Komatsu, K., Koyanagi, T., Nemoto, T., Ohsugi, Y., Michi, Y., Katagiri, S., Takeuchi, Y., Ishihara, K., & Iwata, T. (2021). Non-surgical treatment for periodontitis and peri-implantitis: Longitudinal clinical and bacteriological findings-a case report with a 7-year follow-up evaluation. *SAGE Open Medical Case Reports*, *9*. https://doi.org/10.1177/2050313X211029154

Shimchuk, A. A., Weinstein, B. F., & Daubert, D. M. (2021). The impact of a change in classification criteria on the prevalence of peri-implantitis: A cross-sectional analysis. *Journal of Periodontology*, *92*(9), 1339–1346. https://doi.org/10.1002/jper.20-0566

Sousa, V., Nibali, L., Spratt, D., Dopico, J., Mardas, N., Petrie, A., & Donos, N. (2017). Peri-implant and periodontal microbiome diversity in aggressive periodontitis patients: A pilot study. *Clinical Oral Implants Research*, *28*(5), 558–570. https://doi.org/10.1111/clr.12834

Sun, F., Wei, Y., Li, S., Nie, Y., Wang, C., & Hu, W. (2022). Shift in the submucosal microbiome of diseased peri-implant sites after non-surgical mechanical debridement treatment. *Frontiers in Cellular and Infection Microbiology*, *12*, 1091938. https://doi.org/10.3389/fcimb.2022.1091938

Tong, Y., Zheng, L., Qing, P., Zhao, H., Li, Y., Su, L., Zhang, Q., Zhao, Y., Luo, Y., & Liu, Y. (2020). Oral microbiota perturbations are linked to high risk for rheumatoid arthritis. *Frontiers in Cellular and Infection Microbiology*, *9*, 475. https://doi.org/10.3389/fcimb.2019.00475

Tsigarida, A., Chochlidakis, K., Fraser, D., Lampraki, E., Einarsdottir, E. R., Barmak, A. B., Papaspyridakos, P., & Ercoli, C. (2020). Peri-implant diseases and biologic complications at implant-supported fixed dental prostheses in partially edentulous patients. *Journal of Prosthodontics*, *29*(5), 429–435. https://doi.org/10.1111/jopr.13165

Tsigarida, A. A., Dabdoub, S. M., Nagaraja, H. N., & Kumar, P. S. (2015). The influence of smoking on the peri-implant microbiome. *Journal of Dental Research*, *94*(9), 1202–1217. https://doi.org/10.1177/0022034515590581

van der Horst, J., Buijs, M. J., Laine, M. L., Wismeijer, D., Loos, B. G., Crielaard, W., & Zaura, E. (2013). Sterile paper points as a bacterial DNA-contamination source in microbiome profiles of clinical samples. *Journal of Dentistry*, *41*(12), 1297–1301. https://doi.org/10.1016/j.jdent.2013.10.008

Wang, Q., Lu, H., Zhang, L., Yan, X., Zhu, B., & Meng, H. (2020). Peri-implant mucositis sites with suppuration have higher microbial risk than sites without suppuration. *Journal of Periodontology*, *91*(10), 1284–1294. https://doi.org/10.1002/JPER.19-0634

Yan, Z., Yang, F., Cao, J., Ding, W., Yan, S., Shi, W., Wen, S., & Yao, L. (2021). Alterations of gut microbiota and metabolome with Parkinson's disease. *Microbial Pathogenesis*, *160*, 105187. https://doi.org/10.1016/j.micpath.2021.105187

Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., & Glöckner, F. O. (2014). The SILVA and "all-species living tree project (LTP)" taxonomic frameworks. *Nucleic Acids Research*, *42*(D1), D643–D648. https://doi.org/10.1093/nar/gkt1209

Yu, X. L., Chan, Y., Zhuang, L., Lai, H. C., Lang, N. P., Leung, W. K., & Watt, R. M. (2019). Intra-oral single-site comparisons of periodontal and peri-implant microbiota in health and disease. *Clinical Oral Implants Research*, *30*(8), 760–776. https://doi.org/10.1111/clr.13459

Zheng, H., Xu, L., Wang, Z., Li, L., Zhang, J., Zhang, Q., Chen, T., Lin, J., & Chen, F. (2015). Subgingival microbiome in patients with healthy and ailing dental implants. *Scientific Reports*, *5*, 10948. https://doi.org/10.1038/srep10948

Zitzmann, N. U., Berglundh, T., Marinello, C. P., & Lindhe, J. (2001). Experimental peri-implant mucositis in man. *Journal of Clinical Periodontology*, *28*(6), 517–523. https://doi.org/10.1034/j.1600-051x.2001.028006517.x

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