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Microbiome in maintained periodontitis and its shift over a single maintenance interval of 3 months

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Abstract

Aim: To assess the subgingival microbial shift of maintained periodontitis treated by ultrasonic scaling (US) or air polishing (AP) during a 3-month maintenance interval.

Materials and methods: We conducted a 12-week randomized split-mouth controlled trial with US and AP in 17 maintained subjects (bleeding on probing [BOP%] ≤25%, probing depth [PD] ≤5 mm). They were monitored at baseline, week 2, week 8 and week 12. The V3-V4 region of the 16S rDNA from 136 subgingival plaque samples was sequenced and analysed.

Results: Treatment by US or AP could effectively reduce the PD, microbial richness, diversity, periodontitis-associated microbiota and pathogenic metabolism in maintained periodontitis. Bacteria recolonized after treatment and returned to the pretreatment level 12 weeks after treatment. Ultrasonic scaling group demonstrated slight advantage in reducing BOP (%), pathogenic bacteria and metabolism than AP group. Pathogenic microbiota and commensal microbiota kept a balance in subgingival community of maintained patients during the 3-month interval.

Conclusions: Treatment by US or AP effectively reduced the pathogenicity of subgingival microbiome by reducing microbial diversity, proportion of periodontitis-associated microbiota and pathogenic metabolism. It helped to keep a balanced subgingival community and stable periodontal condition over a single maintenance interval of 3 months.

KEYWORDS
air polishing, maintenance treatment, microbiology, periodontitis, ultrasonic scaling

1 | INTRODUCTION

Periodontitis is the sixth most prevalent disease worldwide. It affects almost 50% of the world's population and is the primary cause of tooth loss among adults (Albandar, 2005). It has been established that disordered microbial community causes periodontitis, while balanced microbial community maintains a healthy periodontal condition (Abusleme et al., 2013). Some patients with periodontitis regain periodontal health after successful treatment and maintain of a healthy condition for a long term, which may be evidence of a balanced microbial community. However, as compared with periodontally healthy individuals, maintained patients have increased risk of recurrent periodontitis (Chapple et al., 2018; Teles, Patel, Socransky, & Haffajee, 2008). Therefore, even though periodontal therapy has good clinical efficacy, the subgingival microbial community of these patients might be different from that of healthy individuals.

The subgingival microbiota is involved in the onset and progression of periodontitis (Li et al., 2014). An increase in pathogenic microbiota could trigger a potential host inflammatory response that contributes to tissue destruction and attachment loss. The characteristics of the subgingival microbiome in patients with

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periodontitis and its shift after clinical intervention have been investigated. It was demonstrated that patients with periodontitis harboured a more pathogenic microbial community than healthy individuals (Abusleme et al., 2013), and the proportion of periodontitis-associated taxa decreased and the proportion of health-associated taxa increased after periodontal treatment (Liu et al., 2017; Shi et al., 2015). Then, the bacteria recolonized in periodontal pockets. At 3 months after subgingival debridement, the detection frequencies and proportions of pathogenic bacteria were still lower than that of pre-treatment (Sanz-Sanchez, Ortiz-Vigon, Herrera, & Sanz, 2016). And the detection frequencies of periodontal pathogens rebounded to almost the same level (Lu, Feng, Xu, & Meng, 2012) or over than that of pre-treatment at 6 months post-treatment (Beikler et al., 2004). The maintained individuals received successful periodontal treatment and maintained periodontal homeostasis for a long time, which may be the evidence of a balanced subgingival microbial community. However, do maintained patients who previously had periodontitis have the same microbial community as healthy individuals? Haffajee et al. (Haffajee et al., 1998) compared the subgingival microbiota of 35 well-maintained elder subjects and 30 periodontally healthy subjects using checkerboard DNA–DNA hybridization and did not find a significant difference in any species. Conversely, another study by Teles et al. (Teles et al., 2008), using the same method, demonstrated that periodontal pathogens in the maintained subjects remained significantly higher when compared with healthy subjects. Therefore, the characteristics and it shift of subgingival microbial community in maintained subjects still need to be determined. High-throughput sequencing of the 16S rRNA gene could provide deep insight into the composition of the oral microbiome, which could give us a global view of microbial shift during maintenance period.

Maintenance care is definitively required to remove dental plaque biofilm and to prevent recurrence of periodontitis after active therapy (Axelsson & Lindhe, 1981; Pihlstrom, 2014). Patients receiving proper treatment can sustain a long-term healthy periodontal condition during maintenance therapy. Long-term longitudinal studies demonstrated that maintenance treatment could effectively reduce tooth-loss rate, reduce the cost of dental treatment and improve patients' quality of life (Axelsson, Nystrom, & Lindhe, 2004). Ultrasonic scaling (US) and air polishing (AP) are two main approaches during maintenance therapy as part of guided biofilm therapy (GBT) (Wennstrom, Dahlen, & Ramberg, 2011). And they are both safe, comfortable and effective for periodontal maintenance therapy (Petersilka, 2011, Wennström, Tomasi, Bertelle, & Dellasega, 2005). In addition, it has been confirmed that periodontal maintenance therapy every 3 months could maintain long-term healthy periodontal homeostasis for patients with periodontitis after active treatment (Ramfjord, 1987).

Hence, the objective of this present investigation is to identify the shift of the subgingival bacterial community after treatment with US or AP over a single maintenance interval of 3 months.

Clinical Relevance

Scientific Rationale for Study: Some patients with periodontitis regain and keep healthy periodontal condition with scheduled maintenance therapy after successful periodontal treatment. However, they still have a higher risk of recurrent periodontitis. Therefore, scheduled and effective maintenance treatment is necessary. This study was to evaluate the microbial shift after treatment by ultrasonic scaling or air polishing during a 3-month interval.

Principal Findings: The longitudinal study demonstrated that ultrasonic scaling or air polishing could help maintained patients keep a balanced microbial community and maintain a healthy periodontal condition during a 3-month maintenance interval.

Practical Implications: Maintained patients still have a high risk of recurrent periodontitis, and scheduled treatment by ultrasonic scaling or air polishing is effective for periodontal healthcare. This present study could provide essential baseline data of subgingival microbiome for future applications in assessing the effect of periodontal treatment for maintained periodontitis.

2 | MATERIALS AND METHODS

This was a 12-week prospective interventional study. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Peking University School and Hospital of Stomatology (approval number: IRB00001052-05106). The clinical study was registered at Chinese Clinical Trial Registry (approval number: ChiCTR-INR-17013073). All participating patients were given information about the study and signed informed consent before the inclusion.

2.1 | Participants

Maintained subjects imply that they had received regular periodontal maintenance and exhibited minimal evidence of disease progression and gingival inflammation. They were selected from a longitudinal trial performed at the Department of Periodontology, Peking University School and Hospital of Stomatology. The maintained patients recruited in this study had received regular periodontal maintenance care every 3–6 months for 2.5–24.5 years (mean 14.2 years) after successful periodontal therapy consisting of plaque control, scaling and root planning, as well as surgery if it was necessary. They had maintained a periodontally stable condition with more than 20 teeth, BOP (%) ≤25%, PD ≤ 5 mm (wisdoms and distal aspects of second molar are not included) and exhibited minimal evidence of disease progression and gingival inflammation.

TABLE 1 Demographic characteristics and clinical parameters of patients with maintained periodontitis at baseline and week 12

	Ultrasonic scaling group		Air polishing group	
	Baseline	Week 12	Baseline	Week 12
Age, years (mean ± *SD*)	50.18 ± 12.08			
Male (%)	41.18%			
No. of missing teeth (mean ± *SD*)	1.00 ± 1.12		0.88 ± 0.90	
Plaque index, median (Q1, Q3)	0 (0, 1)	0 (0, 1)	0 (0, 1)	0 (0, 1)
Probing depth (mm, mean ± *SD*)	2.49 ± 0.29	2.39 ± 0.28[a]	2.53 ± 0.31	2.39 ± 0.32[a]
Bleeding on probing (BOP %)	20.50	17.44[a]	20.84	18.84

Note: Paired comparisons were analysed using paired Student's *t* test or Wilcoxon signed-rank test.
[a]Significant differences between baseline and week 12. No significant difference was found between ultrasonic scaling group and air polishing group.

2.2 | Clinical monitoring and sample collection

Clinical periodontal examination including plaque index (PLI), PD and BOP was performed at baseline and 12 weeks after treatment. To minimize interindividual variability, the randomized split-mouth method was adopted. The left half-mouth and the right half-mouth were divided into the US group and AP group according to a computer-generated randomized number. Clinical interventions were performed as previously described (Lu, He, Zhao, & Meng, 2018).

Maintained individuals were sampled at four time points: baseline (before maintenance treatment) and 2 weeks, 8 weeks and 12 weeks after maintenance treatment (Figure S1). Subgingival plaque samples at the mesiobuccal sites of two mandibular first molars (if the first molar was lost, the mandibular second molar would be investigated) were collected separately by sterile curettes.

2.3 | Sequencing processing and analysis pipeline

The clinical parameters between baseline and 12 weeks, US group and AP group of the subjects were analysed using paired Student's *t* test (normal distribution) or Wilcoxon signed-rank test (non-normal distribution).

The genomic DNA of bacteria was isolated as previously described (Lu et al., 2018). 16S rRNA gene V3-V4 was amplified using 338F/806R universal primer. Library preparation and sequencing was performed on the Illumina MiSep PE300 platform. The sequencing data were processed using QIIME v 1.9.1 (Caporaso et al., 2012) pipeline. After raw sequences were trimmed and filtered, the remaining high-quality sequences with a similarity threshold of 97% were clustered as an operational taxonomic unit (OTU). Then, sequences were annotated separately against the Human Oral Microbiome Database (HOMD) (Dewhirst et al., 2010).

Alpha diversity, taxa at all levels, bacterial types and metabolism were evaluated using paired nonparametric tests (Wilcoxon signed-rank test) for significance test of match groups comparisons. The microbial differences between shallow pockets and medium pockets were evaluated by Mann–Whitney test. Principle coordinate analysis (PCoA) was performed to examine the robustness of group-wise clustering using Bray–Curtis distance based on OTU (Bray & Curtis, 1957). A heatmap was created to show the distribution of genera during the maintenance interval. Spearman correlation was performed to evaluate the co-occurrence network with core genera (relative abundance > 1%). Results were visualized using software based on R 3.5.0 and Python 3.7.0.

3 | RESULTS

The demographic and clinical characteristics of 17 maintained patients are presented in Table 1, and the distribution of sites with different periodontal depth are presented in Table S1. The clinical parameters of the US group and AP group were homogeneous at baseline. Probing depth in both group and BOP (%) in US group significantly decreased at week 12 after maintenance treatment, comparing with baseline ($p < .05$).

A total of 4,672,387 high-quality sequences were acquired, with an average of 34,355 sequences per sample (ranging from 18,447–61,147). Clustering of all high-quality sequences at 97% identify resulted in 834 OTUs. In total, 13 phyla, 28 classes, 46 orders, 82 families, 156 genera and 395 species were detected in the subgingival microbiome.

3.1 | Microbiological shift after maintenance therapy with US or AP during a maintenance interval

The microbial richness presented by Chao 1 in US group was significantly reduced at 2 weeks after treatment ($p < .05$; Figure 1a). The AP group exhibited similar change, and the richness significantly increased from week 2 to week 8 and week 12 ($p < .05$). At week 2, the richness in the AP group was higher than that in US group. The microbial diversity presented by Shannon in the US group significantly

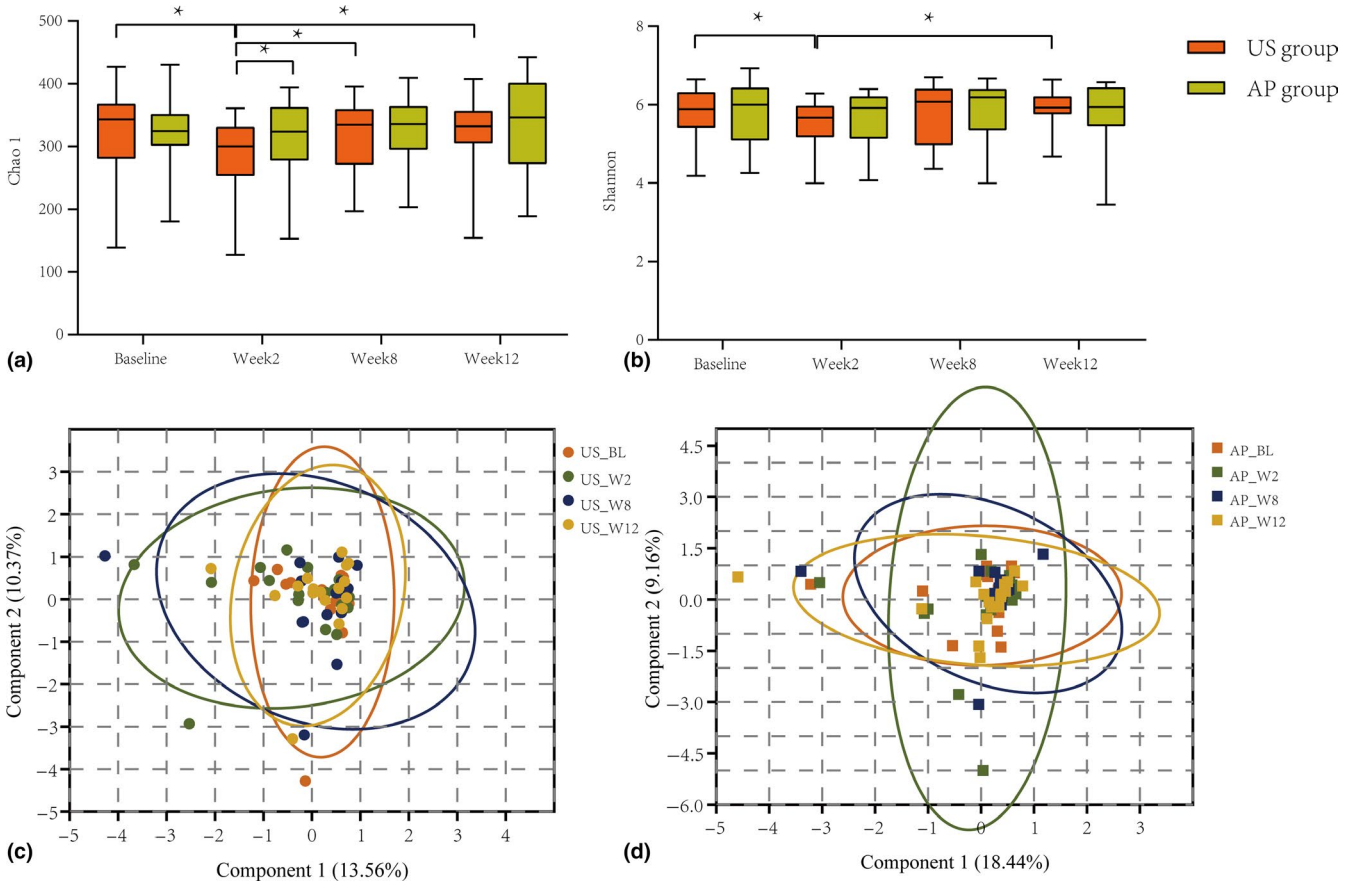

FIGURE 1 The shift of subgingival microbiome during the 12-week maintenance duration within the US group and AP group. (a) Microbial richness at four time points within the two groups presented by Chao 1. (b) Microbial diversity at four time points within the two groups presented by Shannon. (c) PCoA by Bray–Curtis distance between baseline, week 2, week 8 and week 12 in the ultrasonic scaling group. (d) PCoA by Bray–Curtis distance between baseline, week 2, week 8 and week 12 in the air polishing group. US, ultrasonic scaling group; AP, air polishing group; PCoA, principle coordinate analysis; BL, baseline; W2, 2 weeks; W8, 8 weeks; W12, 12 weeks

decreased after treatment at week 2 and then increased at week 12 subsequently ($p < .05$; Figure 1b). No significant difference between the US group and AP group was found at baseline or week 12. The PCoA plot revealed close distribution of microbial communities among baseline, 2 weeks, 8 weeks and 12 weeks within US group and AP group (Figure 1c, d).

3.2 | Transition of microbial composition after maintenance therapy with US or AP during a maintenance interval

The distribution of phylum at four time points in US group and AP group was presented in the barplot, and five major bacteria account for relatively balanced proportion (Figure 2). The line chart showed that three phylum, *Saccharibacteria* TM7, *Spirochaetes* and *Synergistetes*, significantly decreased after treatment and increased afterward (Figure 2b). The distribution of the top 40 genera in the maintained patients was presented with a heatmap (Figure 2c). The genera of *Actinomyces* and *Streptococcus* were two predominant components in subgingival microbiome with a relative abundance greater than 10%, and followed by subpredominant genus of *Leptotrichia Capnocytophaga*, *Lautropia*, *Fusobacterium* and *Neisseria* with relative abundance > 5% all over the 12-week duration. Several pathogenic genera such as *Treponema* and *Bacteroidaceae* have significantly decreased, and some beneficial genera such as *Streptococcus* and *Serratia* have significantly increased. The comparisons at class, order, family and species level also showed significant reduction of pathogenic microbiota and increase of commensal microbiota after treatment by US or AP (Figure S2). The longitudinal reduction of pathogenic taxa in US group was more significant than AP group such as *Saccharibacteria* TM7 G-5 and *Olsenella*.

3.3 | The changes of bacterial types and metabolism after maintenance therapy with US or AP during a maintenance interval

The bacteria were compared according to the classification of oxygen requirements, staining characteristics and shape of bacteria (Figure 3a-f). The bacterial types by oxygen requirements and staining characteristics did not showed significant change from baseline to week 12, while the proportions of bacteria with different shapes significantly changed. In US group, the proportion of rod-shaped

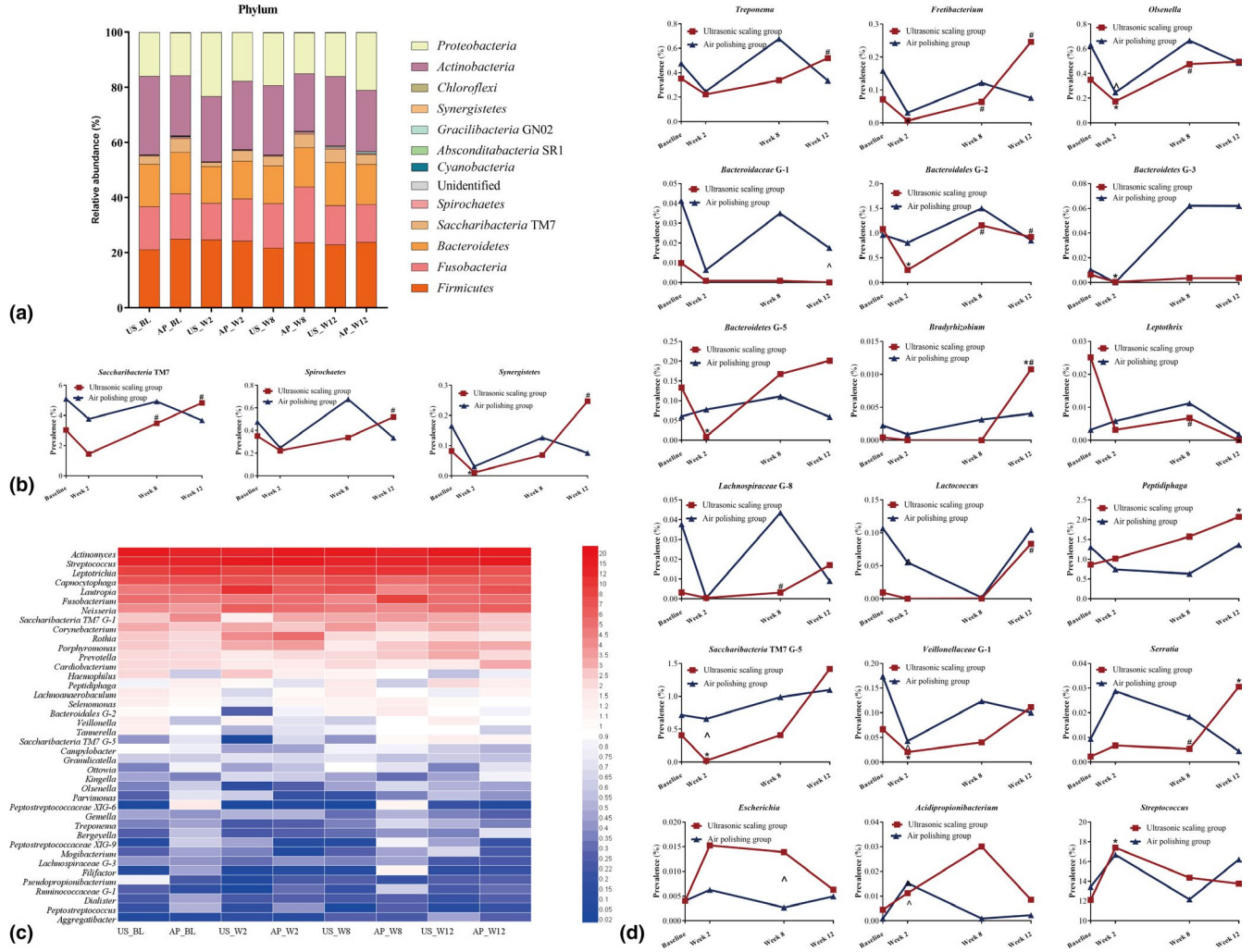

FIGURE 2 Microbial shift during the 12-week maintenance interval in US group and AP group. (a) The distribution of phylum in the maintained condition at four time points within two groups by heatmap at the genera level. (b) The change of phylum presented by line chart. (c) The distribution of top 40 genera in the maintained condition at four time points within two groups by heatmap at the genera level. (d) The change of genera presented by line chart. Tested by Wilcoxon rank-sum test. *Significantly different from baseline with $p < .05$. #Significantly different from week 2 with $p < .05$. ^Significant differences between US group and AP group with $p < .05$. US, ultrasonic scaling group; AP, air polishing group

bacteria significantly decreased after treatment, and coccus-shaped bacteria significantly increased after treatment and decreased at week 8 and week12. Spiral-shaped bacteria also slightly increased from week 2 to week 8. In AP group, coccus-shaped bacteria showed an increase from week 2 to week 8, and spiral-shaped significantly decreased after treatment and increased subsequently. There was no significant difference in bacterial types between US group and AP group at four time points.

Bacterial metabolism also showed some changes during the 12-week maintenance interval (Figure 3g). Some pathways involving in advanced cell activities such as transcription factors and protein processing in endoplasmic reticulum have significantly decreased after treatment, while pathways involving in basic cell mobility and metabolism consisting of bacterial motility proteins and secretion system have significantly increased after treatment. Furthermore, some pathways involving in bacterial pathogenesis such as bacterial chemotaxis and bacterial invasion of epithelial cells have decreased after treatment and increased subsequently. In addition, the metabolism differences between US group and AP group mainly existed at week 2. AP group harboured higher abundance of carbohydrate metabolism, while US group harboured higher abundance of lipid metabolism and amino acid metabolism.

3.4 | The association between microbiota and periodontal depth

To evaluate the association between microbiota and periodontal depth, the taxa at all levels were compared in periodontal pockets ≤ 3 mm and 4–5 mm (Figure 4).

Health-associated microbiota, including family of *Lachnospiraceae XIV*, genus of *Lachnoanaerobaculum*, species of *Lachnoanaerobaculum saburreum* and *Actinomyces odontolyticus*, were significantly higher

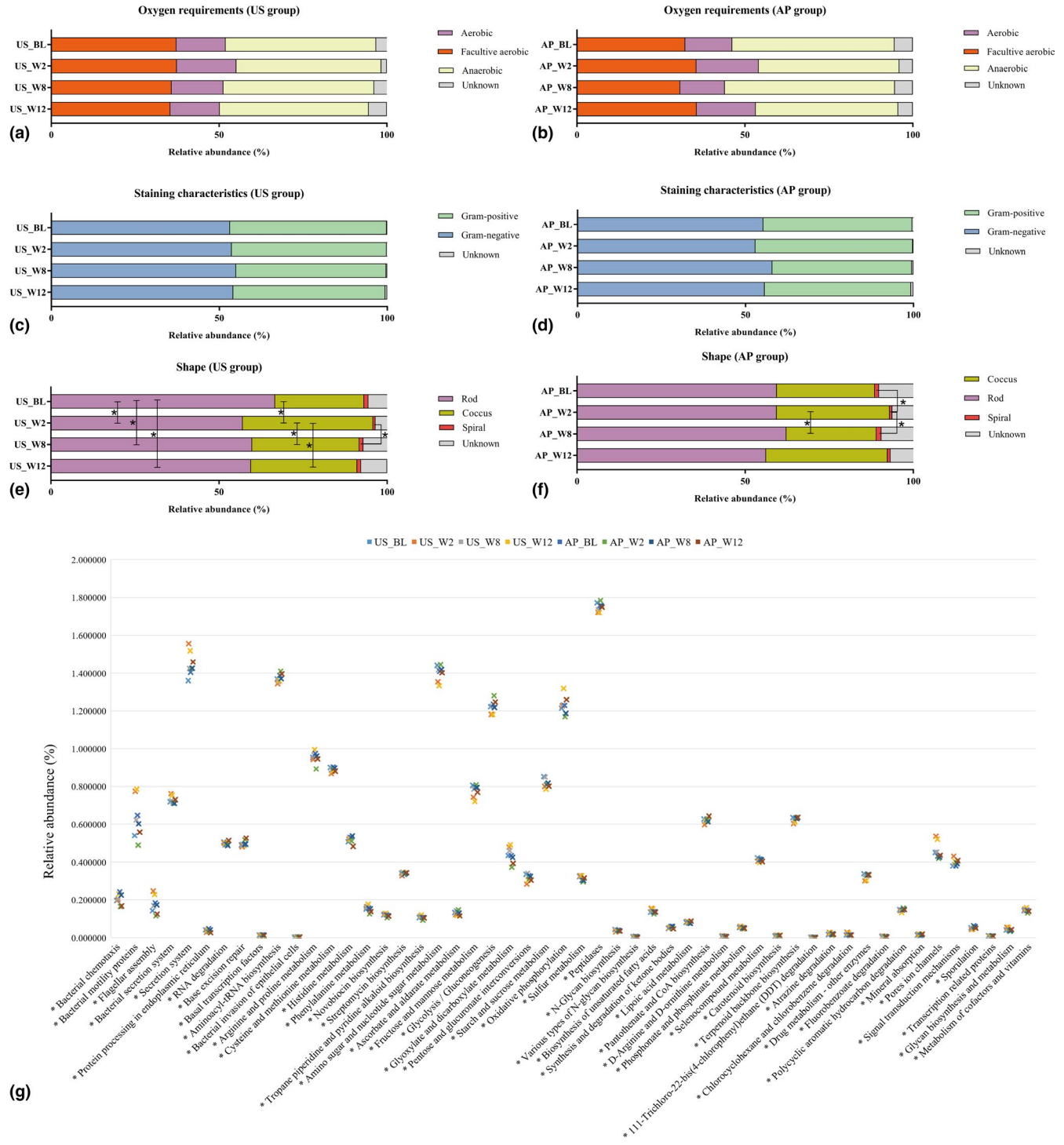

FIGURE 3 The shift of bacterial types and metabolism. (a) The distribution of oxygen requirements in US group. (b) The distribution of oxygen requirements in AP group. (c) The distribution of staining characteristics in US group. (d) The distribution of staining characteristics in AP group. (e) The distribution of shape in US group. (f) The distribution of shape in AP group. (g) Differences in bacterial metabolism. *$p < .05$ tested by Wilcoxon rank-sum test. US, ultrasonic scaling group; AP, air polishing group; BL, baseline; W2, 2 weeks; W8, 8 weeks; W12, 12 weeks

in shallow pockets (≤ 3mm) than in medium pockets (4–5 mm), while periodontitis-associated microbiota, including *Prevotella* 300, *Prevotella* 475, *Treponema* 237, *Prevotella* 515 and *Selenomonas* 501, were significantly lower in shallow pockets (≤3 mm) than in medium pockets (4–5 mm).

3.5 | Microbiome profile of maintained periodontitis

The co-occurrence network showed the symbiotic relationship among core genera with relative abundance > 1% (Figure 5). The positive correlation diagram presented two main interleaving related

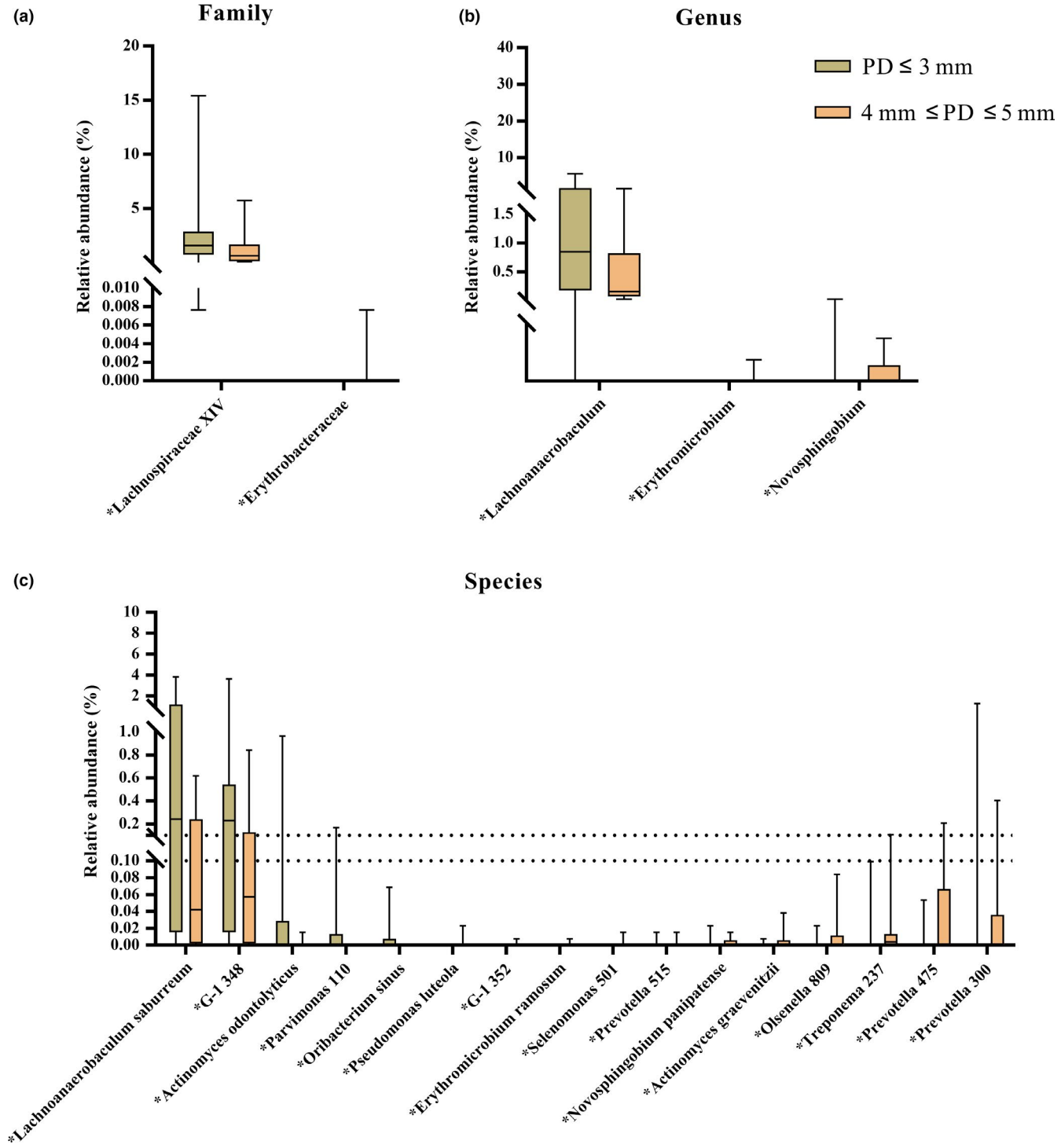

FIGURE 4 Comparisons of microbial composition between shallow pockets (PD ≤ 3 mm) and medium pockets (4-5 mm). Tested by Mann–Whitney *U* test

clusters: pathogenic genera such as *Porphyromonas* were strongly associated with each other and commensal genera such as *Streptococcus* were also strongly associated with each other (Figure 5a). Two clusters were linked by the genera of *Capnocytophaga*, *Cardiobacterium* and *Neisseria*. The two clusters showed similar relative abundance and strength of symbiotic relationship, which might indicate a balanced microbial community in maintained patients.

The negative correlation diagram was clearly divided into two groups, and the taxa of the two groups had multiple negative relationships with each other (Figure 5b). Interestingly, pathogenic

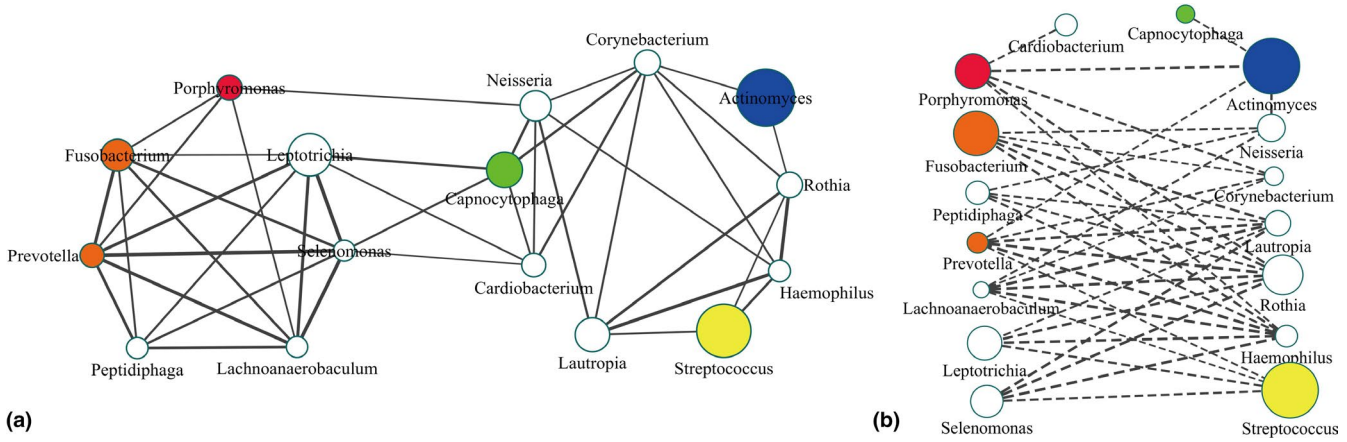

FIGURE 5 The co-occurrence network of core genera. The co-occurrence network of core genera (relative abundance > 1%) tested by Spearman with $p > .05\%$. (a) Positive symbiotic network; (b) negative symbiotic network. The size of the nodes was determined by relative abundance. The thickness of the connecting lines was determined by correlation coefficient. The colour of the nodes was determined by the complexes to which they belonged to, as described by Socransky (Socransky et al., 1998)

genera, such as *Porphyromanas*, *Fusobacterium* and *Prevotella*, were assigned to one side, while beneficial genera, such as *Streptococcus*, *Actinomyces* and *Neisseria*, were assigned to the other side. This might indicate the antagonistic relationship between pathogenic microbiota and beneficial microbiota in the subgingival community.

4 | DISCUSSION

This present study demonstrated that maintained individuals with history of periodontitis could keep a balanced subgingival microbiome during a single maintenance of 3 months after treatment by ultrasonic scaling or air polishing. The two approaches effectively reduce microbial richness, microbial diversity and pathogenic microbiota and increase beneficial microbiota during maintenance period, resulting in a return to previous levels at 12 weeks. However, the microbial structure and core composition remained constant during the 12-week maintenance interval.

The advantage of this study is that it provides a preliminary global-scale framework of subgingival communities in maintained periodontitis, which could help us to understand the characteristics and its shift during a maintenance interval. However, 16S rRNA sequencing is limited when trying to analyse the pathogenicity of a specific taxa. It only tells us who is there, but does not tell how pathogenic or beneficial they are. Microbiota belonging to the same genera or species might have distinct virulence factors eliciting periodontitis. Another advantage lays in the design of randomized split-mouth controlled trial which minimized the impact of individual differences including age, gender, systemic condition, genetic susceptibility, habits, clinical condition and oral hygiene, thereby increasing the power of the study compared to whole-mouth design. Potential weakness is that split-mouth design might cut down the microbial differences between US group and AP group. Theoretically, saliva as a reservoir pool of bacteria could mobilize microorganisms throughout the oral cavity and might have influence on subgingival microbiome. However, subgingival microbiome is site-specific which is mainly affected by periodontal depth and local inflammation (Ge, Rodriguez, Trinh, Gunsolley, & Xu, 2013; Shi et al., 2018). In addition, tight gingiva and narrow gingival sulcus with gingival crevicular fluid flowing out continuously in maintained patients could protect the subgingival microenvironment from saliva.

BOP is an important indicator for periodontal stability (Lang, Adler, Joss, & Nyman, 1990). Ramseier et al. (Ramseier et al., 2015) conducted a longitudinal cohort study on Swiss population and demonstrated the BOP stability thresholds were BOP% <16% in smokers (n = 121 patients) and < 23% in non-smokers (n = 201 patients) or former smokers (n = 123 patients). However, Joss et al. (Joss, Adler, & Lang, 1994), in a retrospective study conducted in a private clinic, revealed that a frequency of 25% of sites with BOP can be considered a limit among individuals with periodontal stability over a 4-year period as well as among individuals with the progression of periodontitis over the same period of time. In addition, patients recruited in this study did not show clinical progression of periodontitis over a maintenance duration of 2.5–24.5 years (mean 14.2 years) (Zhao, He, & Meng, 2015). Therefore, we adopted BOP < 25% as threshold of periodontal stability.

Maintenance treatment by US and AP showed significant microbiological effect with reduced microbial richness and diversity, which indicated a less complex and more evenly distributed community after treatment. Furthermore, the change of bacterial composition and metabolism after treatment reduce the pathogenicity of subgingival community to periodontal tissue. Both US and AP demonstrated decrease of the proportion of pathogenic microbiota and increase of the proportion of commensal microbiota after treatment, which was consistent with previous studies tested by checkerboard DNA–DNA hybridization (Kargas, Tsalikis, Sakellari, Menexes, & Konstantinidis, 2015; Wennstrom et al., 2011). Bacteria recolonized after treatment and returned to the pre-treatment level 12 weeks after treatment, which is similar to that reported before (Lu et al., 2018; Reinhardt et al., 2019). As for metabolic pathways,

the advanced bacterial activities and pathogenic functions also have significantly decreased after maintenance treatment, while relative abundance of basic cell mobility increased which demonstrated less pathogenic to periodontal tissue. Ultrasonic scaling had slight advantage in terms of disturbing the subgingival community with sharper decrease of pathogenic microbiota and pathways, which has also been validated in previous study (Moene, Decaillet, Andersen, & Mombelli, 2010). Altogether, maintenance treatment by ultrasonic scaling or air polishing could effectively reduce the pathogenicity of subgingival community and keep stable periodontal condition during the 12-week interval.

During the 12-week maintenance interval, the subgingival microbiome of maintained patients was dominated by genera *Actinomyces*, *Streptococcus*, *Leptotrichia*, *Capnocytophaga*, *Lautropia*, *Fusobacterium* and *Neisseria*, which was less pathogenic than that in periodontitis as reported before (Boutin et al., 2017; Chen et al., 2018; Li et al., 2015). When comparing with healthy subjects reported previously, the pathogenic components were slightly higher in maintained periodontitis, which might expose maintained individuals to a higher risk of recurrent periodontitis (Griffen et al., 2012). This may also provide an explanation as to the epidemiological results demonstrating that although treatment of patients with periodontitis has good clinical efficacy, they still have a higher susceptibility of recurrence as compared with periodontally healthy subjects (Teles et al., 2008). *Actinomyces* and *Streptococcus*, as the early-stage congregation core, and *Fusobacterium*, as the late-stage coaggregation core, constitute the main part of the subgingival community (Kolenbrander & London, 1993). Previous studies reported that *Leptotrichia*, *Fusobacteria* and *Capnocytophaga* were linked to periodontitis (Abusleme et al., 2013; Griffen et al., 2012; Li et al., 2014; Zhou et al., 2014) and some of their species belonged to the orange or green complex as described by Socransky (Socransky, Haffajee, Cugini, Smith, & Kent, 1998). These complexes represent moderate periodontal pathogenic bacterium and mainly play a bridging role between aerobic bacterium and anaerobic bacterium, providing the red complex a suitable anaerobic environment for colonization (Kolenbrander et al., 2002). This study might be helpful for understanding the susceptibility of maintained patients with a history of periodontitis. Therefore, close monitor and scheduled maintenance treatment should still be provided to maintained individuals with a history of periodontitis.

The complexity of the subgingival microbiome has been recognized and described by Socransky (Socransky et al., 1998). However, that study lacked the consideration of beneficial taxa. Increasing evidence has suggested that it is dysbiosis of the microbial community that causes the onset and progression of periodontitis (Abusleme et al., 2013; Duran-Pinedo et al., 2014). The lack of a beneficial organism in a biofilm may be just as important in the contribution to disease as the presence of a pathogen. Our study demonstrated an intricate antagonistic relationship between two distinct groups of bacterial organisms—beneficial taxa and pathogenic taxa, which is in agreement with previous studies about periodontitis (Shi et al., 2015). Adhesins, nutrition and metabolism might count for the synergistic relationship, and lactose-inhibitable congregations and quorum sensing might count for the antagonistic relationship among genera (Ben Amara et al., 2018; Kolenbrander & London, 1993; Polak, Shapira, Weiss, & Houri-Haddad, 2012; Rovai & Holzhausen, 2017). The underlying mechanisms must be investigated in future studies.

It highlights that ultrasonic scaling and air polishing are two effective approaches to keep a relative balanced subgingival community during the 12-week maintenance interval. Air polishing is more time-saving, comfortable and effective in removing biofilm, however, limited in removing dental calculus, while ultrasonic scaling might have an advantage in removing biofilm and calculus simultaneously (Zhao et al., 2015). This study could help periodontists or hygienists to choose proper method for periodontal healthcare. In addition, this present study could provide essential baseline data of subgingival microbiome for future applications in assessing the effect of periodontal treatment for maintained periodontitis. Further studies should consider integrating microbiome of maintained patients into the predictive models of periodontal diseases.

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CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflict of interests in connection with this article.

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SUPPORTING INFORMATION

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