The prevalence of periodontopathogenic bacteria in saliva is linked to periodontal health status and oral malodour

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This study investigated whether an improvement in periodontal health resulted in changes in the prevalence of periodontopathogenic bacteria in saliva and tongue coatings and a reduction in volatile sulfur compounds (VSCs: H_2S and CH_3SH) linked to oral malodour. The subjects were 35 patients who visited the breath odour clinic of Kyushu Dental College, Japan. Their mean age was 51.2 ± 18.3 years (mean $\pm$ sd). A clinical examination performed at baseline and 2 months after periodontal treatment assessed VSCs in mouth air using gas chromatography, periodontal probing depth and bleeding on probing (BOP) in all subjects; saliva and tongue coatings were also collected. Genomic DNA was isolated from the samples, and the proportions of five periodontopathogenic bacteria (*Porphyromonas gingivalis*, *Tannerella forsythensis*, *Treponema denticola*, *Prevotella intermedia* and *Prevotella nigrescens*) were investigated using quantitative real-time PCR. The subjects were classified into four groups based on the presence of a periodontal pocket of more than 4 mm (PD) and VSCs above the organoleptic threshold level (VSCT) as follows: –PD/–VSCT group, subjects without PD or VSCT; –PD/+VSCT group, those without PD but with VSCT; +PD/–VSCT group, those with PD but without VSCT; and +PD/+VSCT group, those with PD and VSCT. Although the mean PD values in the +PD/–VSCT and +PD/+VSCT groups, BOP in the +PD/+VSCT group, and H_2S and CH_3SH concentrations in the –PD/+VSCT and +PD/+VSCT groups were greater than in the other groups at baseline, we found no significant difference among the four groups after periodontal treatment. The proportion of periodontopathogenic bacteria in saliva was higher in the +PD/–VSCT and +PD/+VSCT groups than in the –PD/–VSCT and –PD/+VSCT groups at baseline and after treatment, but the proportions of bacteria in saliva after treatment were reduced compared to the baseline. Furthermore, the differences in the proportions of the five target bacteria in the tongue coating were not as apparent as those in saliva at baseline or after treatment. The prevalence of periodontopathogenic bacteria in saliva may reflect periodontal health status and influence VSC levels in mouth air.

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INTRODUCTION

Periodontitis, a polymicrobial mixed infection and a major oral disease (Socransky & Haffajee, 2005), is thought to be a risk factor for oral malodour because periodontopathogenic bacteria have a high ability to produce volatile sulfur compounds (VSCs) including hydrogen sulfide (H_2S) and methyl mercaptan (CH_3SH) (Persson *et al.*, 1990; Yaegaki & Sanada, 1992b). The H_2S and CH_3SH in mouth air are prominent elements of oral malodour and are produced primarily through the putrefactive activities of bacteria

present in saliva, in periodontal pockets, on the tongue surface and in other areas (Bosy *et al.*, 1994; Miyazaki *et al.*, 1995; Tonzetich, 1977).

Furthermore, increased tongue biofilm as assessed by observable tongue coating contributes to oral malodour more than other related factors, such as increased periodontal pockets or poor oral hygiene (Miyazaki *et al.*, 1995). Moreover, the tongue has been reported to be even more malodorous in subjects with periodontitis (Mantilla Gomez *et al.*, 2001; Yaegaki & Sanada, 1992b). Recently, tongue microbiota has been the focus of oral malodour research, and several studies have reported a relationship between periodontal pathogens in the tongue biofilm coating and oral malodour (Tanaka *et al.*, 2004; Washio

Abbreviations: BOP, bleeding on probing; GC, gas chromatograph; PD, probing depth of more than 4 mm; VSC, volatile sulfur compound; VSCT, VSCs above the organoleptic threshold level.

et al., 2005). Washio *et al.* (2005) reported that periodontal disease-associated bacteria, such as *Porphyromonas gingivalis* and *Prevotella intermedia*, may not be associated with oral malodour in patients without periodontal disease or with low-to-moderate levels of oral malodour, whilst Tanaka *et al.* (2004) reported that periodontal pathogens on the tongue dorsa can contribute greatly to VSC production. Additionally, several studies have indicated a relationship between micro-organisms present on the tongue and those present in saliva; it has been suggested that a relationship exists between oral malodour and periodontal pathogens in saliva, in addition to those in the tongue coating (Awano *et al.*, 2002; Kozlovsky *et al.*, 1994). However, it remains unclear whether the diversity of periodontal pathogens in saliva is associated with the production of oral malodour as well as those in the tongue coating.

Generally, differences in the prevalence of periodontal pathogens in the tongue coating and saliva are thought to be related to periodontal health conditions (Kozlovsky *et al.*, 1994; Mantilla Gomez *et al.*, 2001; Umeda *et al.*, 1998). Thus an improvement in periodontal health may be linked to reductions in periodontal pathogens in saliva and the tongue coating, with improvements in oral malodour. We sought to investigate whether the prevalence of periodontal pathogens in tongue coating and saliva was related to periodontal health conditions and oral malodour, and whether improved periodontal health resulted in changes in the prevalence of periodontopathogenic bacteria in saliva and the tongue coating, with a reduction in oral malodour.

METHODS

Subject population. The subjects were 35 patients (8 males, 27 females) who visited the breath odour clinic of Kyushu Dental College, Japan, from 2004 to 2006. Their mean age ($\pm$ SD) was 51.2 ± 18.3 years. Subjects with medical disorders or who had taken antibiotics or other antimicrobial therapy in the previous 3 months and pregnant women were excluded.

Informed consent was obtained from each subject. The protocol was reviewed by the ethics committee of Kyushu Dental College.

Study design. Subjects were selected randomly from patients who complained of oral malodour. At baseline, they underwent a clinical examination, which included measurement of the concentrations of H_2S and CH_3SH in mouth air, periodontal probing depth of six sites per tooth, verification of bleeding on probing (BOP) and evaluation of the tongue coating. Furthermore, resting saliva and tongue coating were sampled to evaluate the prevalence of periodontal bacteria following the measurements of H_2S and CH_3SH . After clinical examination, they received oral hygiene instruction at baseline and underwent initial periodontal therapy, such as scaling and professional mechanical teeth cleaning, followed twice more at 2 and 4 weeks after baseline, whilst all subjects were prohibited from tongue cleaning during the 2 months until the second assessment. Two months from baseline, the parameters assessed at baseline were re-evaluated. All examinations and samplings were performed by a trained dentist.

Clinical examination. The H_2S and CH_3SH concentrations in mouth air were determined using a gas chromatograph (GC) (G2800; Yanaco) equipped with a flame photometric detector and a $3.4 \text{ mm} \times 3 \text{ m}$ glass column packed with 25 % 1,2,3-Tris(2-cyanoethoxy)propane in chromosorb W (AW-DMCS, 60/80 mesh), as described previously (Awano *et al.*, 2002). Briefly, after closing the lips for 30 s, 10 ml mouth air was collected in the sample loop of the multi-port injector connecting to the GC equipment and immediately injected into the GC with the carrier gas (nitrogen gas) by switching the multi-port injector from the sample loading port to the sample injection port.

The number of sites with a periodontal probing depth of 4 mm or greater or with BOP was scored. Tongue coating scores were estimated by accumulated thickness and were defined as follows: 0, no coating apparent; 1, less than one-third of the area of tongue dorsum coated; 2, between one-third and two-thirds of the surface coated; 3, more than two-thirds of the surface coated (Miyazaki *et al.*, 1995).

Sampling and preparation of saliva and tongue coating. Saliva samples were collected by spitting resting saliva into sterile plastic tubes for 5 min. After removing the saliva on the lingual surface using a three-way syringe, tongue coat samples from a 1 cm^2 area of the thickest tongue biofilm on the posterior tongue dorsum were collected by scraping two or three times, using the cap of a sterile microcentrifuge tube. All samples were stored at -30°C after collection.

Genomic DNA from the saliva sample (100 μl) and the tongue coat sample was isolated using an Easy DNA kit (Invitrogen) according to the manufacturer's instructions, and was used as template for real-time PCR.

Real-time PCR. The species-specific primers and TaqMan probes for five periodontopathogenic bacteria (*Porphyromonas gingivalis*, *Tannerella forsythensis*, *Treponema denticola*, *Prevotella intermedia* and *Prevotella nigrescens*) are shown in Table 1. Initially, conventional PCR was used to confirm the presence of each of these bacteria in the saliva and tongue coating of each subject, and real-time PCR was then performed using the specimens in which their presence had been confirmed. Amplification and detection were performed using a LightCycler Sequence Detection System (Roche Diagnostics). Each PCR was performed as described previously (Kato *et al.*, 2005). For relative quantification, the copy number of each target bacterial gene was normalized to the copy number of the 16S rRNA gene using a simplified comparative threshold cycle method (Yoshida *et al.*, 2003).

Statistical analysis. Subjects were divided into four groups on the basis of the existence of periodontal pockets with a probing depth of more than 4 mm (PD) and volatile sulfur compounds in mouth air at or above the organoleptic threshold level (VSCT: concentration of $H_2S \geq 1.5 \text{ ng per } 10 \text{ ml air}$ or $CH_3SH \geq 0.4 \text{ ng per } 10 \text{ ml air}$) as follows: -PD/-VSCT group, patients without PD and VSCT; -PD/+VSCT group, patients without PD and with VSCT; +PD/-VSCT group, patients with PD and without VSCT; and +PD/+VSCT group, patients with PD and VSCT.

Comparisons among the four groups were assessed by analysis of variance and a post-hoc test, and those between baseline data and follow-up data were performed using a paired Student's *t*-test. Comparisons of the proportions of the five periodontopathogenic bacteria compared with total bacterial cells in saliva and tongue coating were evaluated using the Kruskal-Wallis test and the Mann-Whitney U-test among the four groups, and Wilcoxon's rank-sum test between baseline and follow-up data. Correlations between the proportion of each periodontopathogenic bacterium and total bacterial cells in saliva and tongue were analysed using Spearman's

Table 1. Oligonucleotide primers and probes used for real-time PCR

Primer/probe	Sequences (5'→3')*	Amplicon size (bp)	Reference
Primer			
<i>Porphyromonas gingivalis</i> -F	TACCCATCGTCGCCTTGGT	126	Kato <i>et al.</i> (2005)
<i>Porphyromonas gingivalis</i> -R	CGGACTAAAACCGCATACACTTG		
<i>Tannerella forsythensis</i> -F	ATCCTGGCTCAGGATGAACG	225	Suzuki <i>et al.</i> (2004)
<i>Tannerella forsythensis</i> -R	TACGCATACCCATCCGCA		
<i>Treponema denticola</i> -F	AGAGCAAGCTCTCCCTTACCGT	105	Yoshida <i>et al.</i> (2004)
<i>Treponema denticola</i> -R	TAAGGGCGGCTTGAAATAATGA		
<i>Prevotella intermedia</i> -F	TGTCGGTTTACTGGCTATGTTCTC	117	Yoshida <i>et al.</i> (2005)
<i>Prevotella intermedia</i> -R	CTGTCTGTGTGGCCATCTTGAAG		
<i>Prevotella nigrescens</i> -F	CAGCCAAACACGTACCTGTTG	150	Yoshida <i>et al.</i> (2005)
<i>Prevotella nigrescens</i> -R	TTCCATTGGACACATCAGCATT		
Universal-F	CGCTAGTAATCGTGGATCAGAATG	69	Yoshida <i>et al.</i> (2003)
Universal-R	TGTGACGGGCGGTGTGTA		
Probe			
<i>Porphyromonas gingivalis</i>	FAM-TCGAGTATTCTCAAGCATTCTCGCACG-TAMRA		
<i>Tannerella forsythensis</i>	FAM-ACTTTGCTCCCAAGTAACATGGAACACGAG-TAMRA		
<i>Treponema denticola</i>	FAM-CAGCGTTCGTTCTGAGCCAGGATCA-TAMRA		
<i>Prevotella intermedia</i>	FAM-TCAAAGACGCACGTACCAATCCAGACC-TAMRA		
<i>Prevotella nigrescens</i>	FAM-TGGCTAACCAATATCTGCACGCTGCCT-TAMRA		
Universal	FAM-CACGGTGAATACGTTCCCGGGC-TAMRA		

*FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine.

rank correlation coefficient. All analyses were performed using SPSS software (version 11; SPSS Japan).

RESULTS AND DISCUSSION

Comparison of patients between baseline and follow-up

Baseline data before treatment and follow-up data after treatment for the patients and the four groups, based on the existence of PD and VSCT, are shown in Table 2. In the baseline data, the mean ages in the +PD/-VSCT and +PD/+VSCT groups, which were composed of patients with PD, were significantly higher than those in the two groups without PD. A recent study reported that probing depth increased in prevalence with increasing age, and levelled off at around 50 years of age and above (Susin *et al.*, 2005). This study also suggested that the number of subjects with PD tended to increase with increasing age. The number of teeth in patients in the +PD/-VSCT group was significantly lower than in the -PD/-VSCT group [Fisher's least significant difference (LSD) test, $P < 0.05$]. Furthermore, no significant difference in numbers of PD was observed between the +PD/-VSCT and +PD/+VSCT groups at baseline, whilst the number of BOP in the +PD/+VSCT group was significantly greater than those in the other groups (Fisher's LSD test, $P < 0.05$). Thus periodontal health status in patients with PD and VSCT at baseline got worse, with increases in the number of sites with BOP, compared with patients with PD alone (without VSCT). Furthermore, the mean scores of tongue

coating were significantly greater in the +PD/+VSCT group than in the other groups. Thus increased tongue coating may be related to deterioration of periodontal health with an increase in both PD and BOP, and an increase in tongue biofilm coating may be associated with oral malodour, as in previous reports (Bosy *et al.*, 1994; Miyazaki *et al.*, 1995; Yaegaki & Sanada, 1992a).

A previous study reported that the CH₃SH:H₂S ratio in mouth air from patients with PD was greater than in control subjects (Yaegaki & Sanada, 1992b). The mean concentrations of H₂S and CH₃SH at baseline were significantly higher in both the -PD/+VSCT and +PD/+VSCT groups compared with the -PD/-VSCT and +PD/-VSCT groups. The concentration of CH₃SH was significantly higher in the +PD/+VSCT group than in the -PD/+VSCT group in addition to the two groups without VSCT (Fisher's LSD test, $P < 0.05$). These results indicated that the existence of PD was related not only to the production of H₂S, but also to production of CH₃SH, and that the character of oral malodour in patients with PD differed from that of patients without PD.

In the follow-up data, after treatment, the numbers of PD in the +PD/-VSCT and +PD/+VSCT groups, BOP in the -PD/-VSCT and +PD/+VSCT groups, the mean score of tongue coating in the +PD/+VSCT group and the concentration of H₂S in the -PD/+VSCT and +PD/+VSCT groups and CH₃SH in the +PD/+VSCT group were all significantly reduced compared with those at baseline (paired *t*-test, $P < 0.05$). Moreover, no significant difference was detected in any parameter among the four

Table 2. Comparison of patients at baseline (before treatment) and follow-up (after treatment), for all patients and the four groups, classified based on the existence of a periodontal pocket of 4 mm or more (PD) and volatile sulfur compounds in mouth air at or above the organoleptic threshold level (VSCT) [mean (standard deviation)]

Results are shown as mean % ($\pm$ SD). *n*, Number of patients (number of females); tongue coating, mean score of tongue coating; H₂S, mean concentration of hydrogen sulfide; CH₃SH, mean concentration of methyl mercaptan.

Characteristic	Sampling time	Group				
		Total [<i>n</i> =35 (27)]	–PD/–VSCT [<i>n</i> =11 (9)]	–PD/+VSCT [<i>n</i> =5 (5)]	+PD/–VSCT [<i>n</i> =12 (8)]	+PD/+VSCT [<i>n</i> =7 (5)]
Age (years)		51.1 (18.2)	37.1 (15.1)	49.0 (17.8)	61.7 (13.7)*	56.6 (18.0)*
Number of teeth		24.6 (4.3)	26.8 (1.9)	25.4 (1.9)	22.9 (5.2)†	23.3 (6.0)
Number of PD	Baseline	6.1 (13.1)	0.0 (0.0)	0.0 (0.0)	9.3 (11.3)†	14.9 (23.3)*
	Follow-up	1.5 (3.9)‡	0.0 (0.0)	0.0 (0.0)	1.5 (2.2)‡	5.1 (7.6)‡
Number of BOP	Baseline	21.7 (19.6)	18.4 (22.1)	10.2 (6.9)	19.7 (15.4)	38.4 (20.8)§
	Follow-up	7.5 (10.0)‡	1.9 (2.3)‡	1.0 (1.7)	13.9 (13.2)	10.1 (7.6)‡
Tongue coating	Baseline	1.3 (0.5)	1.0 (0.4)	1.4 (0.5)	1.3 (0.5)	1.9 (0.4)§
	Follow-up	1.1 (0.4)‡	0.9 (0.3)	1.0 (0.0)	1.2 (0.5)	1.1 (0.4)‡
H ₂ S (ng per 10 ml air)	Baseline	1.5 (2.1)	0.4 (0.4)	3.7 (3.3)	0.2 (0.2)	5.1 (2.4)
	Follow-up	0.7 (0.9)‡	0.4 (0.5)	0.5 (0.2)‡	0.2 (0.2)	1.0 (0.9)‡
CH ₃ SH (ng per 10 ml air)	Baseline	0.4 (0.8)	0.0 (0.0)	0.5 (0.4)*	0.0 (0.1)	1.5 (1.1)§
	Follow-up	0.1 (0.4)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.1 (0.1)‡

*P <0.05 versus –PD/–VSCT group and –PD/+VSCT group.

†*P* <0.05 versus –PD/–VSCT group.

‡*P* <0.05 versus baseline data (paired *t*-test).

§*P* <0.05 versus –PD/–VSCT group, –PD/+VSCT group and +PD/–VSCT group.

||*P* <0.05 versus –PD/–VSCT group and +PD/–VSCT group (all Fisher's LSD test).

groups in the follow-up data. Thus it was evident that the scaling and professional mechanical teeth cleaning that all subjects underwent resulted in improvements in periodontal health and oral malodour.

Comparison of five periodontopathogenic bacteria in saliva between baseline and follow-up

The proportions of the five periodontopathogenic bacteria compared with total bacterial cells in saliva at baseline and follow-up are shown in Table 3. In the baseline data, the mean percentage ($\pm$ SD) of the five periodontopathogenic bacteria in the saliva of all subjects was approximately 3.2 % ($\pm$ 5.5). The proportions of *Porphyromonas gingivalis* and *Treponema denticola* and the total proportion of the five bacteria in the +PD/–VSCT group were significantly higher than in the two groups without PD (Mann–Whitney U-test, *P* <0.05). Furthermore, the proportion of *Treponema denticola* in the +PD/+VSCT group was significantly higher than in the two groups without PD, and the proportions of *Tannerella forsythensis* and all five bacteria in the +PD/+VSCT group were significantly higher than in the –PD/–VSCT group (Mann–Whitney U-test, *P* <0.05). Thus the present findings showed that patients with PD had higher proportions of periodontopathogenic bacteria in saliva compared with those without PD. In particular, *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythensis*, referred

to as the 'red complex' and regarded as the main pathogens in periodontitis, had a higher prevalence in patient groups with PD than in those without PD. We reported previously that the presence of *Tannerella forsythensis* in subjects with periodontitis was strongly correlated with the concentration of VSCs in mouth air (Awano *et al.*, 2002). This study also found that the higher prevalence of *Tannerella forsythensis* in patients with PD was associated with enhanced oral malodour. *Tannerella forsythensis* has been identified along with *Porphyromonas gingivalis* in subgingival plaque and saliva samples from subjects with severe periodontal disease (Holt & Ebersole, 2005; Yang *et al.*, 2004). Thus the increase in the proportion of *Tannerella forsythensis* may be related to the severity of periodontal disease, which may increase the VSC level in mouth air.

In the follow-up data after treatment, the total proportion of the five target bacteria, in addition to *Porphyromonas gingivalis* and *Treponema denticola*, in all patients, and the proportions of *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythensis* in the +PD/+VSCT group were significantly reduced compared with the baseline data (Wilcoxon's rank-sum test, *P* <0.05; Table 3). Furthermore, in the follow-up data, *Porphyromonas gingivalis* and *Treponema denticola* were significantly higher in the +PD/–VSCT group than in the two groups without PD as well as at baseline, and the total proportion of the five bacteria at follow-up was significantly

Table 3. Comparison of the proportions of five periodontopathogenic bacteria and total bacterial cells in saliva between baseline and follow-upResults are shown as mean % ($\pm$ SD).

Bacteria	Sampling time	Group				
		Total patients (n=35)	-PD/-VSCT (n=11)	-PD/+VSCT (n=5)	+PD/-VSCT (n=12)	+PD/+VSCT (n=7)
<i>Porphyromonas gingivalis</i>	Baseline*	2.356 (4.650)	0.406 (0.657)	0.179 (0.221)	5.288 (6.608)†	2.199 (4.561)
	Follow-up*	1.226 (2.732)‡	0.221 (0.281)	0.081 (0.096)	2.793 (4.131)†	1.151 (2.432)‡
<i>Treponema denticola</i>	Baseline*	0.264 (0.517)	0.002 (0.006)	0.016 (0.028)	0.428 (0.621)†	0.529 (0.685)†
	Follow-up*	0.102 (0.175)‡	0.000 (0.001)	0.005 (0.009)	0.173 (0.150)†	0.198 (0.270)‡
<i>Tannerella forsythensis</i>	Baseline*	0.412 (1.565)	0.011 (0.020)	0.090 (0.190)	0.142 (0.210)	1.450 (3.112)\$
	Follow-up*	0.189 (0.557)	0.015 (0.029)	0.006 (0.014)	0.139 (0.257)	0.571 (1.059)‡\$
<i>Prevotella intermedia</i>	Baseline	0.019 (0.019)	0.000 (0.000)	0.000 (0.000)	0.010 (0.025)	0.068 (0.163)
	Follow-up	0.007 (0.018)	0.000 (0.000)	0.000 (0.000)	0.001 (0.022)	0.018 (0.029)
<i>Prevotella nigrescens</i>	Baseline	0.174 (0.608)	0.001 (0.003)	0.199 (0.445)	0.071 (0.173)	0.487 (1.183)
	Follow-up	0.027 (0.062)	0.006 (0.011)	0.000 (0.000)	0.038 (0.092)	0.059 (0.062)
All five bacteria	Baseline*	3.227 (5.495)	0.422 (0.652)	0.484 (0.598)	5.942 (7.081)†	4.734 (6.619)\$
	Follow-up*	1.596 (2.918)‡	0.225 (0.319)	0.093 (0.088)	4.146 (1.382)†	1.998 (2.740)†

* $P < 0.05$ (Kruskal–Wallis test).† $P < 0.05$ versus -PD/-VSCT and -PD/+VSCT.‡ $P < 0.05$ versus baseline data (Wilcoxon's rank-sum test).\$ $P < 0.05$ versus -PD/-VSCT (all Mann–Whitney U-test).

higher in the groups with PD than in the groups without PD (Mann–Whitney U-test, $P < 0.05$). Moreover, the proportion of *Tannerella forsythensis* in the +PD/+VSCT group differed significantly from that in the -PD/-VSCT group ($P < 0.05$).

These results showed that the initial periodontal therapy led to a significant reduction in the proportion of periodontal pathogens such as *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythensis* in saliva, in addition to the improvement in periodontal health and oral malodour. However, the tendency for patients with PD at baseline to have a higher proportion of periodontopathogenic bacteria compared with patients without PD was maintained.

Comparison of the five periodontopathogenic bacteria in tongue coating at baseline and follow-up

The proportions of the five periodontopathogenic bacteria compared with total bacterial cells in the tongue coating at baseline and follow-up are shown in Table 4. The mean percentage of the total five periodontopathogenic bacteria in the tongue coating of all patients was approximately one-sixth lower than in saliva. In baseline data, the proportion of *Treponema denticola* in the +PD/+VSCT group was significantly higher than that in the -PD/-VSCT group (Mann–Whitney U-test, $P < 0.05$). Furthermore, in follow-up data, the +PD/+VSCT group had a significantly higher proportion of *Treponema denticola* compared

with the -PD/-VSCT and -PD/+VSCT groups ($P < 0.05$), and a significantly higher proportion of *Prevotella nigrescens* than the other groups. However, no significant difference was observed in the proportion of each bacterium in all patients or among the four groups between the baseline and follow-up data. These results showed that no relationship existed between the proportion of periodontal pathogens in the tongue coating and differences in PD and VSCT. The improvement in periodontal health and oral malodour resulting from the periodontal therapy seemed not to be reflected in the prevalence of periodontal pathogens in the tongue coating.

Correlation between periodontopathogenic bacteria in saliva and tongue coating

Significant correlations were detected between proportions of the same periodontopathogenic bacterium versus total bacterial cells in saliva and tongue coating (Spearman's rank correlation: *Porphyromonas gingivalis*, $r=0.536$, $P < 0.001$; *Treponema denticola*, $r=0.431$, $P < 0.001$; *Tannerella forsythensis*, $r=0.302$, $P < 0.05$; *Prevotella intermedia*, $r=0.440$, $P < 0.001$; *Prevotella nigrescens*, $r=0.420$, $P < 0.001$). Until now, there has been no evidence of a relationship between the prevalence of periodontal pathogens in saliva and in tongue coatings. However, these results suggest that the prevalence of periodontopathogenic bacteria in saliva may be linked to that of the bacteria in the tongue coating, although the proportion of periodontopathogenic bacteria in the tongue coating was considerably lower than in saliva. Therefore, the proportions of the five

Table 4. Comparison of the proportions of five periodontopathogenic bacteria and total bacterial cells in tongue coating for baseline data and follow-up dataResults are shown as mean % ($\pm$ SD).

Bacteria	Sampling time	Group				
		Total patients (n=35)	-PD/-VSCT (n=11)	-PD/+VSCT (n=5)	+PD/-VSCT (n=12)	+PD/+VSCT (n=7)
<i>Porphyromonas gingivalis</i>	Baseline	0.536 (1.587)	0.052 (0.138)	0.081 (0.149)	1.373 (2.745)	0.055 (0.078)
	Follow-up	0.419 (1.189)	0.066 (0.176)	0.003 (0.008)	1.026 (2.119)	0.079 (0.135)
<i>Treponema denticola</i>	Baseline*	0.028 (0.119)	0.001 (0.003)	0.001 (0.001)	0.082 (0.231)	0.030 (0.022)†
	Follow-up*	0.008 (0.025)	0.000 (0.001)	0.000 (0.000)	0.001 (0.038)	0.038 (0.046)‡
<i>Tannerella forsythensis</i>	Baseline	0.001 (0.058)	0.004 (0.010)	0.000 (0.000)	0.000 (0.000)	0.003 (0.006)
	Follow-up	0.000 (0.026)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.003 (0.005)
<i>Prevotella intermedia</i>	Baseline	0.000 (0.003)	0.000 (0.000)	0.000 (0.000)	0.001 (0.005)	0.001 (0.004)
	Follow-up	0.000 (0.003)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.003 (0.008)
<i>Prevotella nigrescens</i>	Baseline	0.007 (0.040)	0.000 (0.000)	0.000 (0.000)	0.000 (0.002)	0.034 (0.087)
	Follow-up*	0.018 (0.064)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.088 (0.126)\$
All five bacteria	Baseline	0.575 (1.618)	0.058 (0.137)	0.083 (0.149)	1.458 (2.800)	0.126 (0.117)
	Follow-up*	0.448 (1.184)	0.067 (0.176)	0.004 (0.008)	1.027 (2.118)	0.213 (0.229)‡

* $P < 0.05$ (Kruskal–Wallis test).† $P < 0.05$ versus -PD/-VSCT.‡ $P < 0.05$ versus -PD/-VSCT and -PD/+VSCT.\$ $P < 0.05$ versus -PD/-VSCT, -PD/+VSCT and +PD/-VSCT (all Mann–Whitney U-test).

periodontopathogenic bacteria in the tongue coating, as well as those in saliva, may be related to the periodontal health and oral malodour status, although this was not observed in our study. One reason may be that the proportion of the five periodontopathogenic bacteria compared with total bacterial cells in the tongue coating was too low to influence the periodontal health status and oral malodour, even in patients with PD and VSCT. Many oral anaerobic bacteria in addition to the five periodontopathogenic bacteria surveyed here can produce H_2S and CH_3SH *in vitro* (Persson *et al.*, 1990). A recent study reported that the predominant microbiota on the tongue dorsa of healthy subjects was different from that on the tongue dorsa of subjects with oral malodour, and that those species most associated with oral malodour were *Atopobium parvulum*, a phylotype (clone BS095) of *Dialister*, *Eubacterium sulci*, a phylotype (clone DR034) of the uncultivated phylum TM7; *Solobacterium moorei*; and a phylotype (clone BW009) of *Streptococcus* (Kazor *et al.*, 2003). Thus the level of VSCs produced by the tongue coating may reflect differences in the prevalence of other bacteria with a high ability to produce VSCs, rather than the five periodontopathogenic bacteria examined here.

In another recent report, the numbers of total colonies and black/grey colonies sampled from the tongue coating in the odour group were significantly higher than those in the no/low odour group (Washio *et al.*, 2005). Moreover, an increase in the number of H_2S -producing bacteria, such as *Veillonella*, *Actinomyces* and *Prevotella* species, in the tongue biofilm was associated with oral malodour, even though the bacterial

composition of the tongue biofilm was similar in the odour and the no/low odour groups (Washio *et al.*, 2005). In this study, although the amounts of the five target bacteria in the tongue coating were not evaluated, it was evident that increased tongue coating was associated with VSC levels. Thus the difference in the amount of total bacteria in the tongue coating rather than the composition of microflora may influence the production of VSCs more strongly.

This study did not focus on the microflora in subgingival plaque as the diversity of the microbiota is influenced by differences in the sampling sites in addition to differences among subjects (Paster *et al.*, 2001). The bacterial population in subgingival plaque is associated with the local periodontal health status of the sampling site (Haffajee *et al.*, 1998); however, the bacterial population in subgingival plaque sampled from a local site may not reflect a comprehensive periodontal evaluation of a subject. Furthermore, it has been reported that it is useful to observe the prevalence of periodontal pathogens in saliva and tongue coating in addition to in subgingival plaque (Tanner *et al.*, 2006; Umeda *et al.*, 1998). Therefore, it may be more reasonable to use saliva and tongue coating than plaque sampled from the local site to comprehensively investigate the relationship between periodontal health status and the prevalence of periodontal pathogens in the whole mouth.

Conclusions

In this study, we found that the prevalence of the periodontal pathogens *Porphyromonas gingivalis*,

Treponema denticola and *Tannerella forsythensis* in saliva was related to periodontal health status and VSC levels in mouth air, but no relationship was seen between the prevalence of these periodontal pathogens in the tongue coating and the status of periodontal health or VSC levels in mouth air. Moreover, an improvement in periodontal health resulted in lower prevalences of *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythensis* in saliva, with reduced VSC levels in mouth air. Thus our findings suggest that the prevalence of these periodontal pathogens in saliva is linked to periodontal health status and influences VSC levels in mouth air.

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