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Comprehensive microbiological findings in peri-implantitis and periodontitis

Tatsuro Koyanagi[1,2], Mitsuo Sakamoto[2], Yasuo Takeuchi[1], Noriko Maruyama[1,2], Moriya Ohkuma[2] and Yuichi Izumi[1,3]

[1]Department of Periodontology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan; [2]Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Center, Tsukuba, Ibaraki, Japan; [3]Global Center of Excellence (GCOE) Program, International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo Medical and Dental University, Tokyo, Japan

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Abstract

Aim: The microbial differences between peri-implantitis and periodontitis in the same subjects were examined using 16S rRNA gene clone library analysis and real-time polymerase chain reaction.

Materials and methods: Subgingival plaque samples were taken from the deepest pockets of peri-implantitis and periodontitis sites in six subjects. The prevalence of bacteria was analysed using a 16S rRNA gene clone library and real-time polymerase chain reaction.

Results: A total of 333 different taxa were identified from 799 sequenced clones; 231 (69%) were uncultivated phylotypes, of which 75 were novel. The numbers of bacterial taxa identified at the sites of peri-implantitis and periodontitis were 192 and 148 respectively. The microbial composition of peri-implantitis was more diverse when compared with that of periodontitis. *Fusobacterium* spp. and *Streptococcus* spp. were predominant in both peri-implantitis and periodontitis, while bacteria such as *Parvimonas micra* were only detected in peri-implantitis. The prevalence of periodontopathic bacteria was not high, while quantitative evaluation revealed that, in most cases, prevalence was higher at peri-implantitis sites than at periodontitis sites.

Conclusions: The biofilm in peri-implantitis showed a more complex microbial composition when compared with periodontitis. Common periodontopathic bacteria showed low prevalence, and several bacteria were identified as candidate pathogens in peri-implantitis.

Key words: biofilm; dental implants; microbiology; peri-implant infection; peri-implant microbiota; periodontal disease

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Conflict of interest and source of funding statement

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Peri-implant complications have become more common with the expansion of implant treatment for managing tooth loss (Mombelli & Lang 1998, Quirynen et al. 2002, Roos-Jansaker et al. 2006a,b). Implant failure may be classified as early or late. Early failures occur during the healing phase (before osseointegration), and late failures arise subsequent to functional loading after addition of prosthetics. Early failures are associated with a number of patient-related factors such as smoking, bone quality, systemic disease. Late failures (i.e. peri-implantitis and peri-implant mucositis) are inflammatory diseases leading to destruction of supporting tissues around implants after osseointegration (Lindhe et al. 1992, Pontoriero et al. 1994, Esposito et al. 1998, Zitzmann & Berglundh 2008).

Factors associated with late failure of implants are less well understood and reported to be the result of an

imbalance between bacterial challenge and host response (Esposito et al. 1998, Tonetti 1998, Berglundh et al. 2002, Quirynen et al. 2002). After the installation of titanium implants, rapid colonization of bacteria has been observed at the peri-implant sulcus (Van Winkelhoff et al. 2000). Numerous studies have reported increased numbers of total bacteria and higher detection frequencies of periodontopathic bacteria at peri-implant sites (Mombelli et al. 1987, Augthun & Conrads 1997, Leonhardt et al. 1999, Botero et al. 2005, Shibli et al. 2008), while Leonhardt et al. (1999) reported that less common oral species, such as staphylococci, enteric species and yeasts, are recovered from failing implants. These findings indicate the complexity of microbiota in peri-implantitis, and the species responsible for peri-implantitis remain unclear. It is also possible that unknown bacteria are involved. Remaining teeth may also act as a bacterial reservoir, and the composition of microbiota is influenced by the surrounding environment. Several researchers have demonstrated similarities in colonized bacteria between failing implants and surrounding teeth in the same mouth (Papaioannou et al. 1996, Quirynen et al. 1996, Sumida et al. 2002).

Most studies have examined only periodontopathic bacteria, and few studies have focused on the differences in overall bacterial composition. We recently obtained plaque samples from three patients and examined the microbiota of peri-implantitis using the 16S rRNA gene clone library technique (Koyanagi et al. 2010). We found that biofilm in peri-implantitis showed more complex microbiota than that in periodontitis, although the number of samples was limited, and our results did not clarify the role of periodontopathic bacteria in peri-implantitis.

After confirming the complexity of microbiota in peri-implantitis, we added samples and identified bacteria by 16S rRNA gene clone library analysis. Quantitative evaluation of three periodontopathic bacteria (*Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*) was also performed to examine their association with peri-implantitis.

Materials and methods

Subjects and clinical examination

Subjects were recruited at Tokyo Medical and Dental University Hospital Faculty of Dentistry, from August 2009 to June 2010. The criteria for entry in the study were having at least one implant with peri-implantitis and one tooth with periodontitis. Six Japanese subjects (five female and one male, mean age; 61.8 years) who were non-smokers and in good general health were enrolled in the study. They had not received any medication, including systemic antibiotics, anti-inflammatory drugs or oral antimicrobial agents within the last 3 months.

Clinical examinations were performed for the selected teeth and dental implants. The following clinical parameters were assessed at six sites per tooth, and at six sites per implant (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual): (1) probing depth (PD), (2) bleeding on probing (BOP), (3) suppuration (SUP), and (4) Gingival Index (GI) (Mombelli et al. 1987). Intraoral periapical radiographs (Insight dental films; Eastman Kodak Company, SP, Japan) were obtained using the parallel technique. Radiographs were analysed for peri-implant bone loss by the same examiner using smooth components and implant threads as reference points.

Based on clinical and radiographical examination, a diseased implant and a periodontally diseased tooth were selected for plaque sampling in each subject. Diseased implants (implant with peri-implantitis: PI) showed PD $\geq$5 mm with BOP and/or SUP and concomitant radiographic bone loss (bone loss more than three threads, up to half of the implant length). All dental implants for sampling were treated as single prostheses. Periodontally diseased teeth (P) showed PD $\geq$4 mm with BOP and concomitant radiographic bone loss.

The study was approved by the Ethics Committee of Tokyo Medical and Dental University (# 415), and written informed consent was obtained from all subjects.

Sample collection and bacterial DNA isolation

Subgingival plaque samples were obtained from the deepest pockets at the peri-implantitis sites. In addition, samples from the deepest pockets of a periodontally diseased tooth not adjacent to the implant were collected. Thus, two bacterial samples were obtained from each patient. Sampling sites were isolated with sterile cotton rolls. Supragingival plaque was removed with sterile cotton pellets. Three sterile paper points (#45; United Dental Manufactures Inc., Johnson City, TN, USA) were inserted into a pocket until resistance was felt. After 30 s, all three paper points from each sample were placed in a sterile tube with 1 ml of sterile distilled water.

Samples were mixed for 1 min using a vortex mixer. After removing the paper point, each sample was collected by centrifugation at 12,000 *g* for 5 min. The resulting pellet was re-suspended in 150 μl of lysis buffer from a bacterial DNA extraction kit (Mora-extract; AMR Inc., Tokyo, Japan). Samples were then incubated for 10 min at 90°C, and total bacterial genomic DNA was isolated using the Mora-extract kit. Total bacterial DNA was eluted with 200 μl of TE buffer (AMR Inc.) and was stored at −20°C.

16S rRNA gene clone library and real-time PCR analysis

16S rRNA gene clone library analysis was performed as described previously (Sakamoto et al. 2000, 2006). Briefly, the universal primers 27F and 1492R designed based on the 16S rRNA gene were used for PCR amplification (Table 1). PCR mixture (100 μl) contained 10 μl of extracted DNA, 2.5 U of TaKaRa Ex Taq (Takara Bio Inc., Otsu, Japan), 10 μl of 10× Ex Taq buffer, 8 μl of dNTP mixture (0.2 mM each) and 50 pmol of each primer. PCR amplification was performed using a Veriti 200 PCR Thermal Cycler (Applied Biosystems, Foster City, CA, USA) with the following program: 95°C for 3 min; followed by 15 cycles of 95°C for 30 s, 50°C for 30 s and 72°C for 1.5 min; and a final extension period of 72°C for 10 min. PCR products were purified

Table 1. 16S rRNA gene clone library and real-time PCR primers used in this study

Target	PCR primer (5′-3′)*	Position (bp)
Universal 16S rDNA	AGAGTTTGATCMTGGCTCAG	27–1492 (1465)
	TACGGYTACCTTGTTACGACTT	
P. gingivalis 16S rDNA	AGGCAGCTTGCCATACTGCG	729–1132 (404)
	ACTGTTAGCAACTACCGATGT	
T. forsythia 16S rDNA	GCGTATGTAACCTGCCCGCA	120–760 (641)
	TGCTTCAGTGTCAGTTATACCT	
T. denticola 16S rDNA	TAATACCGAATGTGCTCATTTACAT	193–508 (316)
	TCAAAGAAGCATTCCCTCTTCTTCTTA	

*M = A or C; Y = T or C. (Sakamoto et al. 2001).

using a QIAquick PCR purification kit (Qiagen, Valencia, CA, USA).

Purified amplicons were ligated into plasmid vector pCR®2.1 and then transformed into One Shot® INVaF′ competent cells using the Original TA Cloning kit (Invitrogen, San Diego, CA, USA). Plasmid DNAs were prepared using the TempliPhi DNA Amplification Kit (GE Healthcare, Buckinghamshire, UK) from randomly selected recombinants and used as templates for sequencing. Sequencing was conducted using the 27F and 520R primers, a BigDye Terminator Cycle Sequencing kit (Applied Biosystems), and a 3130x Genetic Analyzer (Applied Biosystems).

All sequences were checked for possible chimeric artefacts by the Chimera Check program of the Ribosomal Database Project-II (RDP-II), and were compared with similar sequences of the reference organisms by BLAST search (Altschul et al. 1990). A 16S rRNA gene sequence similarity of 99% was used as the cut-off for positive identification of taxa (operational taxonomic unit: OTU). Less than 99% identity in the 16S rRNA gene sequence was the criterion used to identify bacteria at the species level. Sequences were aligned with the Clustal X 2.0.12 program (Larkin et al. 2007) and were corrected by manual inspection. Nucleotide substitution rates (K_{nuc} values) were calculated (Kimura 1980) after gaps and unknown bases were eliminated. Phylogenetic trees were constructed by the neighbour-joining method (Saitou & Nei 1987). Bootstrap resampling analysis (Felsenstein 1985) was performed to estimate the confidence of tree topologies. Sequences for novel phylotypes were deposited in the DDBJ database under accession numbers AB538407 to AB538428 and AB687684 to AB687713.

Libraries were analysed using the Mothur program v.1.7.2 (Schloss et al. 2009). Distance matrices were calculated using the Dnadist program within the PHYLIP software package version 3.69 (http://evolution.genetics.washington.edu/phylip.html). The Shannon index was used to measure community diversity. The Chao1 index was applied to measure community richness. Principal coordinates analysis (PCA) using UniFrac (Lozupone et al. 2006) was performed to examine the differences in comparisons of bacterial communities.

Real-time PCR was performed as described by Sakamoto et al. (2001), with minor modifications for quantifying three periodontopathic bacteria. Primer sequences are shown in Table 1.

Results

Clinical data on subjects and sites selected for sampling are summarized in Table 2. Subgingival plaque samples were collected from peri-implantitis and periodontitis sites. Mean probing depth of sampling sites was 7.2 ± 3.0 mm (mean ± SD) in peri-implantitis and 6.0 ± 1.5 mm in periodontitis, respectively, and no significant differences were seen (Mann–Whitney *U*-test). A total of 799 clones from 12 samples were subjected to sequence analysis, which revealed 333 species; 231 were uncultivated phylotypes, of which 75 were novel. Of the 333 species, 192 were from PI sites and 148 were from P sites. The numbers of clones/species were higher at PI sites than at P sites in each individual.

All species were classified into 11 phylogenetic groups: *Firmicutes* (45.6%), *Bacteroidetes* (20.1%), *Proteobacteria* (11.0%), *Fusobacteria* (8.4%), *Actinobacteria* (4.5%), *TM7* (4.2%), *Synergistetes* (0.03%), *Spirochetes* (0.02%), *Tenericutes* (0.01%), *Chloroflexi* (0.003%) and *Deferribacteres* (0.003%). The most abundant

Table 2. Characteristics of subjects and clinical data in this study

Subjects	A	B	C	D	E	F
Race	Mongoloid	Mongoloid	Mongoloid	Mongoloid	Mongoloid	Mongoloid
Age (Years)	60	60	60	56	70	62
Gender	Female	Female	Female	Male	Female	Female
Smoking habit	Non	Non	Non	Non	Non	Non
General health	Good	Good	Good	Good	Good	Good
No. natural teeth per subjects	21	20	13	9	24	23
No. implants per subjects	2	6	8	7	2	2
PI site						
Sampling site	⌈7	⌊6	⌊1	⌊4	⌊7	⌈6
Probing depth	5	9	5	7	11	6
Bleeding on probing	+	+	+	+	+	+
Years of implant load	10	7	3	4	8	6
P site						
Sampling site	⌈4	⌊3	⌋4	⌈3	⌋6	⌋4
Probing depth	5	4	4	8	7	7
Bleeding on probing	+	+	+	+	+	+

Table 3. Comparison of diversity and richness of sequenced clones between peri-implantitis and periodontitis sites

Sample source	No. of sequence	No. of OTUs	Shannon index*	Richness†
Peri-implantitis	474	192	4.8(4.7–4.9)	638(451–959)
Periodontitis	325	148	3.7(3.5–3.8)	359(268–520)

*Shannon index and richness are estimated based on 1% differences in nucleic acid sequence alignments. Values in parentheses are 95% confidence intervals, as calculated by the Mothur program.
†Chao1 values, a non-parametric estimate of species richness.

groups were *Firmicutes* and *Bacteroidetes* at both PI and P sites, while the phyla *Chloroflexi* and *Deferribacteres* were only detected at PI sites. Chao 1 richness estimation showed that the clone library of PI sites contained significantly higher 16S rRNA gene diversity than that of P sites. Similarly, the Shannon index was significantly higher at PI sites than at P sites (Table 3). The scatter plot of PCA constructed by the Unifrac distance matrix indicated that microbiota differed between PI and P sites (Fig. 1). Dimensional reduction of the OTU data by PCA explained 43.8% of total variance among the individual samples by the first three components. The greatest component (PC1, 20.1% of variance) discriminated individual specificity. The second greatest component (PC2, 14.2% of variance) discriminated between the samples from periodontitis and peri-implantitis sites.

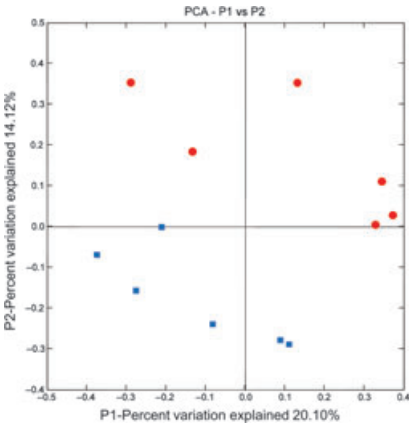

Fig. 1. Principal Coordinates Analysis (PCA) results for all individual samples with OTU clustering of sequences at the 1% difference level. Blue: samples from peri-implantitis; Red: samples from periodontitis. Data were normalized against an equal number of reads per sample, followed by log transformation.

Phylogenetic trees of bacterial species at PI and P sites are shown in Fig. 2a and b. *Fusobacterium* spp. and *Streptococcus* spp. were found at all sampling sites and were predominant at both peri-implantitis and periodontitis sites. In particular, *Fusobacterium nucleatum* was observed at all sites, and overall, it was the most abundant sequence. The genera *Dialister* spp., *Eubacterium* spp. and *Porphyromonas* spp. showed higher prevalence and a greater number of clones at PI sites than at P sites. Although the detection frequency of *Peptostreptococcus* spp. and *Prevotella* spp. was similar between PI (5/6 and 5/6) and P sites (4/6 and 5/6), the number of clones was higher at PI sites in two particular patients. Some species belonging to *Firmicutes*, such as *Parvimonas micra*, *Peptostreptococcus stomatis*, *Pseudoramibacter alactolyticus* and *Solobacterium moorei*, were only observed at PI sites.

The prevalence of *P. gingivalis* was slightly higher at PI sites than at P sites (4/6 versus 2/6). The prevalence of other periodontopathic bacteria, including *T. forsythia* and *T. denticola*, was similar between PI (2/6 and 1/6) and P (3/6 versus 2/6) sites, and all three bacteria were positive at two of six PI sites. *Aggregatibacter actinomycetemcomitans* was only identified at one P site. Furthermore, quantitative evaluation of periodontopathic bacteria revealed that, in most of cases, the numbers of periodontopathic bacteria were slightly higher at PI sites than at P sites (Table 4).

Discussion

Peri-implantitis is a common complication in implant therapy, and at present, management of peri-implantitis is often difficult and unpredictable (Charalampakis et al. 2011). As the primary cause of the disease is considered plaque biofilm around implants, it is worthwhile to determine the types of bacteria present for treatment. The main objective of this study was to characterize the microbial diversity at peri-implantitis sites. Subgingival plaque samples of peri-implantitis and periodontitis were obtained from each patient. Sampling is generally performed using paper points or curettes/scalers. Damage to tissues and implants when curettes/scalers are inserted into subgingival areas is a cause for concern; thus, the paper point method was used in this study.

To our knowledge, few studies have used a 16S rRNA cloning and sequencing strategy to clarify the bacterial diversity of peri-implantitis biofilms. Similarities in microbiota between implants and remaining teeth in partially edentulous subjects is considered to be caused by intraoral translocation of bacteria (Papaioannou et al. 1996, Quirynen et al. 1996, Sumida et al. 2002). According to PCA analysis, interindividual differences were indeed the greatest component, while obvious intraindividual differences in microbiota were observed between PI and P sites. In this study, PI sampling sites showed deeper pockets than P sites, although no significant differences were found. It may be inferred, therefore, that the depth of periodontal pocket influences bacterial composition of biofilms. However in this study, the number of identified bacteria at PI/P sites did not necessarily correlate with the depth of pockets, and moreover, PI sites had a wider variety of bacteria than P sites within each individual (data not shown). The varied chemical and physical properties of teeth and implants, for example, material, surface roughness, surface free energy and presence or absence of implant-abutment configuration, influence the discrepancies in biofilm establishment (Lang & Berglundh 2011).

Firmicutes, *Bacteroidetes* and *Proteobacteria* are known to be major subgingival phyla in periodontitis patients (Kumar et al. 2005, Preza et al. 2009). Our results also confirmed the predominance of *Firmicutes* and *Bacteroidetes* both at PI and P sites, although the two groups differed in the relative proportions of their subgroups. *Dialister* spp.

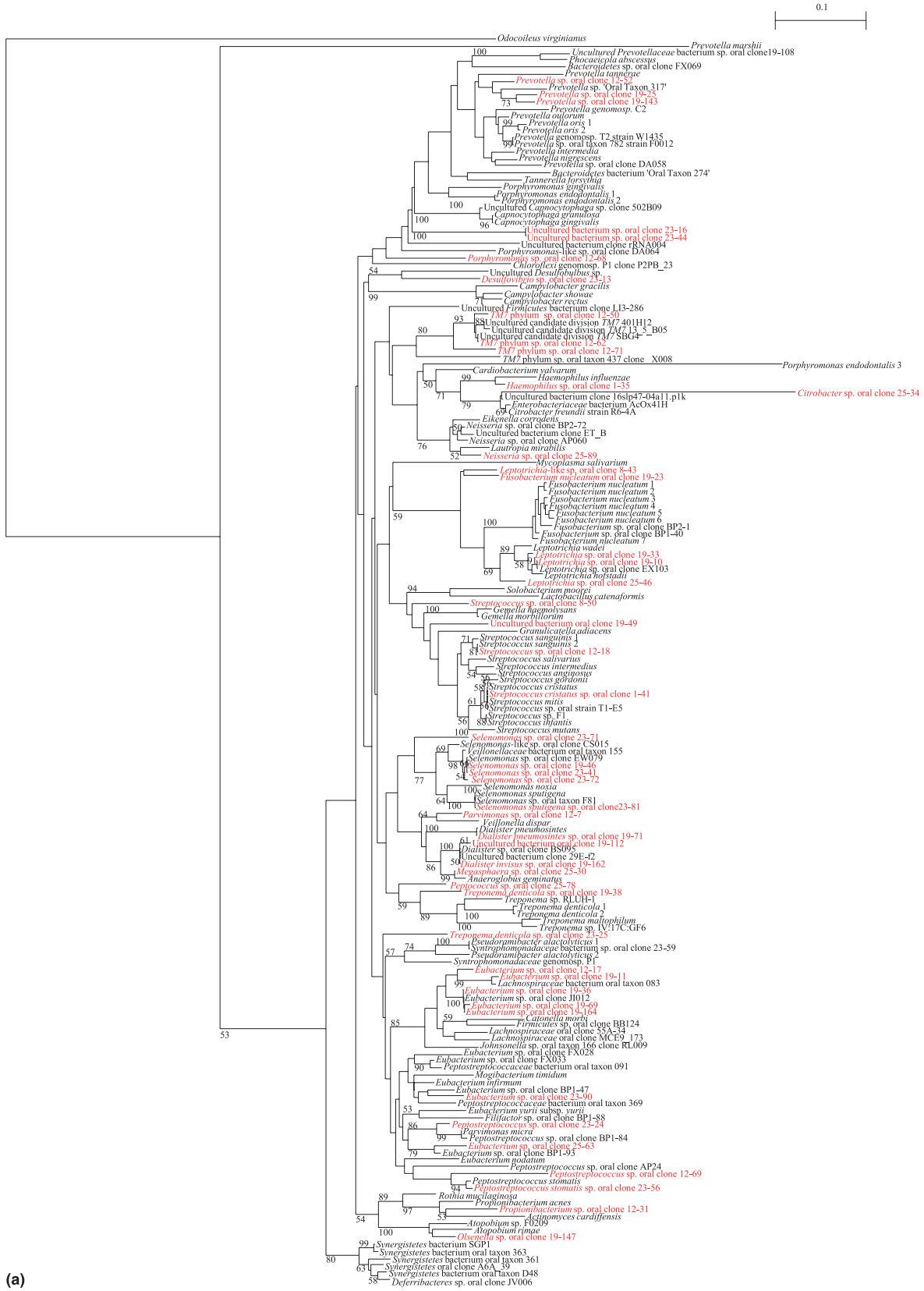

Fig. 2. Phylogenetic tree of bacterial species and phylotypes detected in peri-implantitis (a) and periodontitis (b). Novel phylotypes identified in this study are indicated in red letters. Scale bar represents 0.05 substitutions per nucleotide. Accession numbers for 16S rRNA gene sequences are given for each strain.

Fig. 2. Continued

Table 4. Qualitative and quantitative evaluation of three periodontopathic bacteria in patients with peri-implantitis (PI) and periodontitis (P) by 16S rRNA gene clone library and real-time PCR

Subjects No		*P. gingivalis*		*T. forsythia*		*T. denticola*	
		Clone library	Real time	Clone library	Real time	Clone library	Real time[a]
A	PI	+	–	–	–	–	–
	P	–	–	–	–	–	–
B	PI	+	9.1×10^4	+	1.8×10^3	+	2.1×10^7
	P	+	1.6×10^7	+	9.2×10^4	+	5.5×10^8
C	PI	–	–	–	–	–	–
	P	–	–	–	–	–	–
D	PI	+	3.9×10	+	4.5×10^4	+	1.3×10^7
	P	+	2.4×10	–	–	+	1.3×10^5
E	PI	+	1.7×10^6	–	–	+	3.3×10^8
	P	–	–	–	–	–	–
F	PI	–	–	–	–	–	–
	P	–	–	–	–	–	–

[a]Cells/sample.
Subgingival plaque samples were taken from the deepest pockets of peri-implantitis and periodontitis sites in the same mouth. Prevalence of bacteria was analysed using a 16S rRNA gene clone library. Quantities (cells/sample) were determined by real-time polymerase chain reaction; DNA from known amounts of the bacteria was serially diluted (10^2–10^8 cells) and used as a positive control.

and *Eubacterium* spp. belonging to *Firmicutes* showed high prevalence and proportion at PI sites. *Dialister* spp. are difficult cultivate, and its characteristics are poorly understood. In addition, *Eubacterium* spp. are reportedly associated with periodontitis, although there is limited information (Kumar et al. 2005).

With regard to infection in peri-implantitis, Mombelli & Decaillet (2011) mentioned in their review that the microbiota in peri-implant disease may occasionally show different profiles than those in chronic periodontitis, and this may explain the reports of sporadic high numbers of *Peptostreptococcus* spp. (i.e. *P. micra*) or *Staphylococcus* spp. (i.e. *Staphylococcus aureus* and *Staphylococcus epidermidis*). In particular, *S. aureus* has been shown to have the ability to adhere to titanium surfaces (Harris & Richards 2004) and is often isolated from peri-implant lesions (Alcoforado et al. 1991, Leonhardt et al. 1999, Salvi et al. 2008). In this study, although *Staphylococcus* spp. was not observed at PI sites, *Peptostreptococcus* spp. was more abundant in peri-implantitis. Limited numbers of samples may have reduced the likelihood of species detection.

It is noteworthy that *P. micra* was reportedly identified in a failing implant with inflammation (Alcoforado et al. 1991, Rosenberg et al. 1991), and our results concur with this finding. *P. stomatis* was also found at PI sites, while its characteristics remain unknown. *Peptostreptococus* spp. are commensal organisms in humans that can cause abscesses and necrotizing soft tissue infections (Mombelli & Decaillet 2011), although these bacteria are not aetiological agents of periodontitis. The structure of periodontal tissue surrounding dental implants differs from that surrounding natural teeth, and may be susceptible to even attenuated bacteria.

Although *P. alactolyticus* and *S. moorei* were also only seen in peri-implantitis, there have been few reports, and it is difficult to discuss the role of these species in the aetiology of peri-implantitis. However, a recent study by Colombo et al. (Colombo et al. 2012) showed that *P. alactolyticus* was identified as a species that increased or persisted in high frequency in refractory periodontitis, but was significantly reduced in treatable periodontitis, together with *Bacteroidetes* spp., *P. gingivalis, Prevotella* spp., *T. forsythia, Dialister* spp., *Selenomonas* spp., *Eubacterium* spp., *P. micra, 'Peptostreptococcus* sp. OT113, *Fusobacterium* sp. OT203 or *Streptococcus intermedius*.

Heavy colonization of *Fusobacterium* spp. and *Streptococcus* spp. were observed at both PI and P sites. In particular, *F. nucleatum* was observed at all sampling sites. This bacterium is frequently observed in both peri-implantitis and periodontitis biofilms (Mombelli et al. 1987, Persson et al. 2010) and has the ability to co-aggregate with all of the early and late colonizers (Kolenbrander et al. 1993, Merritt et al. 2009), while extensive intrageneric co-aggregation and production of extracellular polysaccharides by streptococci plays an important role in early plaque formation (Kolenbrander et al. 2002). These two genera appear to play a key role in the development of biofilms on both implants and natural teeth.

Several bacteria, including *A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola* and *T. forsythia*, are considered major aetiological agents of periodontitis and are associated with peri-implant diseases (Hultin et al. 2002). Even in small numbers, these bacteria could be harmful to periodontal tissues. As *A. actinomycetemcomitans* were only present at one periodontitis site in this study, we focussed on quantification of the other three bacteria. These species are the so-called 'red complex' bacteria and are elevated at periodontal disease sites (Socransky 1998). Some of these bacteria were detected at four of six PI sites, with two of six PI sites harbouring all of the red complex bacteria. Their prevalence may be slightly lower when compared with that in chronic periodontitis; we have previously reported that the prevalence of these bacteria is more than 80% in Japanese chronic periodontitis patients (Takeuchi et al. 2003). Although numerous studies have

examined the presence of periodontopathic bacteria at PI sites, the prevalence varies between reports (Mombelli & Decaillet 2011). Generally, periodontal treatment is performed prior to implant placement, and it may affect the prevalence of pathogenic bacteria. Interestingly, there were higher numbers of periodontopathic bacteria at PI sites than on natural teeth. In this study, mean probing depth at PI sites was deeper than that at P sites, although no significant differences were observed. The anaerobic conditions at PI sites may have facilitated the growth of obligate anaerobes. The limited prevalence and/or number of established periodontopathic bacteria at peri-implantitis sites would be reflected by differences in ecological conditions as compared with periodontally affected teeth. Moreover, in this study, a wide variety of bacteria, including uncultivable and unrecognized bacteria, were found around diseased implants. There remains a possibility that unexpected bacteria not related to periodontitis are involved in inflammation around peri-implant tissues.

Most bacteriological studies for peri-implantitis have focussed on specific bacteria (i.e. periodontopathic bacteria). Using the 16S rRNA gene clone library technique, the breadth of bacterial diversity in peri-implantitis was confirmed in this study; the microbiota in peri-implantitis was more complex than that in periodontitis. The prevalence of periodontopathic bacteria was not high, while species such as *Dialister* spp., *Eubacterium* spp. and *Peptostreptococus* spp. were abundant. However, it is premature to draw conclusions about the roles of these bacteria in peri-implantitis and further studies are necessary to verify our results.

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Address:
Yasuo Takeuchi
Department of Periodontology
Graduate School of Medical and Dental Sciences
Tokyo Medical and Dental University
1-5-45 Yushima, Bunkyo-ku,
Tokyo 113-8549
Japan
E-mail: takeuchi.peri@tmd.ac.jp

Clinical Relevance

Scientific rationale for the study: Clinical symptoms of peri-implantitis are similar to those of periodontitis. Although implication of periodontopathic bacteria in peri-implantitis has been reported, few studies have focused on overall bacterial composition.

Principal findings: The composition of microbiota differed between peri-implantitis and periodontitis sites. The genera in *Firmicutes* and *Bacteroidetes* were predominant at both peri-implantitis and periodontitis sites, while these two sites differed in relative proportions of their subgroups. Species such as *Parvimonas micra* and *Peptostreptococcus stomatis* were only seen at peri-implantitis sites.

Practical implications: Bacteriological targets for treating peri-implantitis may differ from those for treating periodontitis.