Transmission of Periodontopathic Bacteria from Natural Teeth to Implants

Masanori Aoki, DDS, PhD;* Kiyotoshi Takanashi, DDS, PhD;* Takashi Matsukubo, DDS, PhD;[†]
Yasutomo Yajima, DDS, PhD;[‡] Katsuji Okuda, DDS, PhD;[§] Toru Sato, DDS, PhD;**
Kazuyuki Ishihara, DDS, PhD[††]

ABSTRACT

Purpose: Prevention of peri-implantitis is essential for the success of implant rehabilitation. Infection by periodontopathic bacteria is a major cause of peri-implantitis. The aim of the present study was to identify the source of peri-implant colonization by periodontopathic bacteria.

Materials and Methods: Twenty-one patients with implants were enrolled in the study. Subgingival plaque samples from the adjacent, occluding, and contralateral natural teeth were collected prior to second-stage surgery. Samples from implant sulci were then obtained 2 weeks later. Detection of periodontopathic bacteria was performed by the polymerase chain reaction.

Results: The detection rates for *Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia*, and *Fusobacterium nucleatum* in all subgingival samples from natural teeth were similar to that in the peri-implant sulci. Multiple logistic regression analysis revealed an association between the detection of *A. actinomycetemcomitans, Prevotella intermedia, Porphyromonas gingivalis, Treponema denticola*, and *F. nucleatum* in the gingival crevices of adjacent teeth and that of the peri-implant sulcus, but no association for *Tannerella forsythia*.

Conclusions: The present findings suggest that colonization by *A. actinomycetemcomitans, Prevotella intermedia, Porphyromonas gingivalis, Treponema denticola*, and *F. nucleatum* at the implant sulcus was affected by these microorganisms in the gingival crevice of adjacent teeth rather than those on occluding and contralateral teeth.

KEY WORDS: implantitis, infection, periodontitis, periodontopathic bacteria, transmission

INTRODUCTION

Implants are used commonly in prosthetic dentistry.[1] Although clinical studies have shown that osseointegration following implant treatment will improve outcomes,[2,3] the procedure still carries the risk of failure. One major cause of failure is peri-implantitis.[4] Implantitis is defined as an inflammatory process affecting the soft and hard tissues around a functioning osseointegrated implant resulting in the loss of supporting bone.[5] The inflammation of soft tissue around an implant and rapid absorption of alveolar bone in peri-implantitis is similar to that observed in chronic periodontitis.[6] Several studies have identified similarities in the pathogenesis of periodontitis and peri-implantitis.[7,8] Poor oral hygiene was reported to be a risk factor for peri-implantitis.[9] It is possible that peri-implantitis is caused by the same mechanism as periodontitis because periodontopathic bacteria have been shown to be involved in implantitis.[10–15] However, non-periodontopathic bacteria such as the *Staphylococcus, Enterococcus*, and *Candida* species have also been detected from peri-implantitis lesions.[13] A higher incidence of peri-implantitis for implants placed in patients with a

*Assistant professor, Department of Crown and Bridge Prosthodontics, Tokyo Dental College, Chiba, Japan; [†]professor and chairman, Department of Epidemiology and Public Health Tokyo Dental College, Chiba, Japan; [‡]professor and chairman, Department of Oral and Maxillofacial Implantology, Tokyo Dental College, Chiba, Japan; [§]professor emeritus, **professor and chairman, Department of Crown and Bridge Prosthodontics, Tokyo Dental College, Chiba, Japan; [††]professor and chairman, Department of Microbiology Tokyo Dental College, Chiba, Japan

Reprint requests: Kazuyuki Ishihara, Department of Microbiology, Tokyo Dental College, 1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan; e-mail: ishihara@tdc.ac.jp

history of chronic periodontitis compared with periodontally healthy subjects has been reported.[16,17] This further suggests the involvement of periodontopathic bacteria in peri-implantitis.

In an earlier report, we showed that the bacteria associated with an implant depended on those present around the natural teeth, and that transmission of periodontopathic bacteria was established at a comparatively early stage.[18] This suggests that peri-implant infection originates from the periodontopathic bacteria surrounding natural teeth although this remains to be confirmed. In order to improve the rate of success of implant treatment, it is essential to clarify the relationship between periodontitis and peri-implantitis and identify the source of infection by periodontopathic bacteria. Such information could provide a breakthrough in the risk assessment of implant treatment, enabling appropriate pretreatment of the surrounding natural teeth. Therefore, we investigated the association between colonization by periodontopathic bacteria in the gingival crevice of natural teeth and that at the sulci of implants to clarify the source of colonization by periodontopathic bacteria.

MATERIALS AND METHODS

Patients and Sampling

Twenty-one patients (5 men and 16 women) who received dental implants at Tokyo Dental College were enrolled in this study (age range: 17–66 years; mean age: 54.6 years). In all cases, tooth loss had resulted from either congenital factors or periodontitis. This study was performed with the permission of the Ethical Committee of Tokyo Dental College. All patients had received periodontal therapy before implant treatment and had adjacent, occluding, and contralateral teeth to the implants. The probing depth of natural teeth in all patients was less than 4 mm. Brånemark Systems (Nobel Bio-Care AB, Göteborg, Sweden), ITI Standard implants (Institut Straumann AG, Basel, Switzerland), and an Ankylos implant (Dentsply Friadent, Mannheim, Germany) were applied in 12, 5, and 5 patients, respectively. None exhibited peri-implantitis during the experimental period.

After informed consent was obtained from all patients, subgingival plaque samples were collected from the natural teeth (adjacent, occluding, and contralateral) and implants with sterile tooth picks. Collection of subgingival plaque samples from the natural teeth was carried out prior to the second-stage surgery. In a previous study,[18] we found that *Porphyromonas gingivalis* displayed a 63.7% detection rate in the implant sulcus 1 month after the second-stage surgery. In addition, previous studies have shown that the recolonization of the subgingival area by microorganisms may occur within 2–8 weeks after treatment.[19–22] Based on these reports, samples from the implant sulci were obtained 2 weeks after the second-stage surgery. The collected plaque samples suspended in TE buffer (pH 8.0) consisted of 10 mM Tris-HCl (Wako Pure Chemical Industries, Osaka, Japan) and 1 mM EDTA (Wako) and microorganisms harvested by centrifugation at 16,000 g at 4°C for 10 minutes. The pellets were then stored at –20°C for subsequent detection of periodontal bacteria.

Detection of Periodontopathic Bacteria by the Polymerase Chain Reaction (PCR) Using Specific Primers Designed from 16s rRNA Sequences. Collected samples were suspended in 100 µl boiling buffer (20 mM Tris-HCl, pH 8.5, 0.5 mM EDTA, 1% Triton X-100) and boiled at 100°C for 10 minutes. Genomic DNA was isolated as described previously.[23] Detection of *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, and *Fusobacterium nucleatum* was performed by PCR as described previously[24,25] in a thermal cycler (Gene Amp PCR system 9700, PE Biosystems, Foster City, CA, USA) using the specific primer pairs listed in Table 1. The PCR products were electrophoresed using 2% agarose gel and then examined under ultraviolet light after staining with Syber Safe DNA stain (Molecular Probe, Eugene, OR, USA).

Statistical Analysis

In the stepwise logistic analysis, the Windows SAS9.1 (SAS Institute, Cary, NC, USA) software was used to evaluate the relationship between colonization by periodontopathic bacteria in the gingival crevises of natural teeth at different positions with that at implants. For each bacterium, we determined the relationship between colonization by each periodontopathic bacteria in the peri-implant sulcus and that in the gingival crevices of adjacent, occluding, or contralateral teeth.

TABLE 1 List of Species-Specific Primers for Polymerase Chain Reaction to Detect Six Periodontopathic Bacteria Targeted

Primer Pairs (5'-3')	Amplicon Length in bp
Aggregatibacter actinomycetemcomitans	557
AAA CCC ATC TCT GAG TTC TTC TTC	
ATG CCAACT TGA CGT TAAAT	
Prevotella intermedia	575
TTT GTT GGG GAG TAAAGC GGG	
TCAACA TCT CTG TGG GCT GCG T	
Porphyromonas gingivalis	404
AGG CGA CTT GCC ATA CTG CG	
ACT GTT AGCAAC TAC CGA TGT	
Treponema denticola	316
TAA TAC CGAAGC TCA TTT ACA T	
TCAAAG TCT CTG TGG GCT GCG A	
Tannerella forsythia	641
GCG TAT GTAACC TGC CCG CA	
TGC TTC AGT GTG AGT TAT ACC T	
Fusobacterium nucleatum	142
CTG AAC ATT GGAAAC TAT ATA GTA	
GAACAA ACAAG	
GTC CTT CAT CGG CTC TTA	
CTA CCT AGG C	

RESULTS

Detection rates of for *A. actinomycetemcomitans, Prevotella intermedia, Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia,* and *F. nucleatum* in the subgingival samples from the natural teeth were 36.5%, 47.6%, 39.7%, 28.6%, 34.9%, and 68.3%, respectively; those from the implant sites were 28.5%, 61.9%, 33.3%, 23.8%, 47.6%, and 76.1%, respectively (Figure 1). The detection rates of these microorganisms were similar between implants and natural teeth.

Detection rates for periodontopathic bacteria at both implant and natural teeth are shown in Table 2. The detection rates of *Porphyromonas gingivalis* and *Treponema denticola* for the adjacent teeth were higher than those at the occluding or contralateral teeth. The detection rate of *Prevotella intermedia* at the adjacent teeth was also higher than that at the occluding teeth, but lower than that at the contralateral teeth. The detection rate of *A. actinomycetemcomitans* at the adjacent teeth was somewhat higher than that at the occluding or contralateral teeth. The detection rates for *Tannerella*

forsythia and *F. nucleatum* at the implants and natural teeth were almost the same.

The relationship between the detection of periodontopathic bacteria around natural teeth and that on implants was statistically analyzed by multiple logistic regression analysis. The results revealed that detection of *A. actinomycetemcomitans, Prevotella intermedia, Porphyromonas gingivalis, Treponema denticola* and *F. nucleatum* from implant sulcus was correlated with detection from the gingival crevises of adjacent teeth. Detection of *Tannerella forsythia* from the peri-implant sulcus showed no association with that from the gingival crevice of natural teeth (Table 3).

DISCUSSION

We investigated the source of colonization by periodontopathic bacteria in peri-implant sulci. Implants are exposed to saliva, which harbors a large number of microorganisms immediately after a surgical procedure. The microbial flora in peri-implantitis lesions is reported to be similar to that in periodontitis.[13,26,27] In the present study, logistic analysis revealed an association between colonization by *A. actinomycetemcomitans, Prevotella intermedia, Porphyromonas gingivalis, Treponema denticola,* and *F. nucleatum* in peri-implant sulci and that by these microorganisms in the gingival crevices of adjacent teeth. In an earlier study, we found that
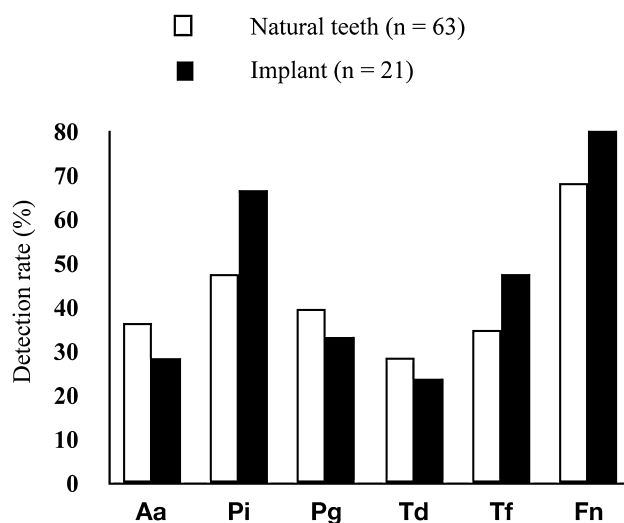

Figure 1 Detection of periodontopathic bacteria from gingival crevice of natural teeth and implant sulci. Natural teeth include the adjacent, occluding, and contralateral teeth relative to the implants. Aa = *Aggregatibacter actinomycetemcomitans*; Pi = *Prevotella intermedia*; Pg = *Porphyromonas gingivalis*; Td = *Treponema denticola*; Tf = *Tannerella forsythia*; Fn = *Fusobacterium nucleatum*.

TABLE 2 Detection Rate of Periodontopathic Bacteria from Both Implants and Adjacent, Occluding, or Contralateral Teeth			
	Teeth		
Species	Adjacent	Occluding	Contralateral
Aggregatibacter actinomycetemcomitans	23.8	19.1	14.3
Prevotella intermedia	38.1	28.6	47.6
Porphyromonas gingivalis	38.1	28.6	28.6
Treponema denticola	19.1	4.8	4.8
Tannerella forsythia	28.6	23.8	28.6
Fusobacterium nucleatum	57.1	61.9	57.1

the detection profiles of *Porphyromonas gingivalis, A. actinomycetemcomitans*, *Treponema denticola*, *Tannerella forsythia,* and *Prevotella intermedia* in peri-implant sulci were similar to those in the gingival crevice of natural teeth.[23] In addition, the same clonal-type of *Porphyromonas gingivalis* or *Prevotella intermedia* were isolated from both peri-implant sulci and the gingival crevices of natural teeth.[18] These data suggest the natural teeth as a major source of the periodontopathic bacteria colonizing at an implant sulcus.

In this study, detection of periodontopathic bacteria from the gingival crevices of occluding and contralateral teeth was not associated with colonization of implant sulci by these microorganisms. Each periodontopathic bacterium has its own mechanism for adhesion. *Porphyromonas gingivalis* was reported to adhere to the tooth surface by the attachment of fimbriae to salivary proline-rich protein and statherin.[28] *Treponema denticola* and *F. nucleatum* colonize dental plaque biofilm by coaggregating with several species of plaque microorganisms.[29–32] It is possible that salivary periodontopathic bacteria released from contralateral and occluding teeth colonize the peri-implant environment. The difference seen in colonization patterns between adjacent, occluding and contralateral teeth may be due to the distance from the original sites of infection. Transmission of microorganisms from adjacent teeth to an implant would not require the release of these microorganisms from dental plaque biofilms. Supragingival plaque biofilms sometimes fuse readily and directly with biofilms on an implant without competition from adhering dental plaque biofilm. Therefore, the high rate of transmission between adjacent teeth and implants observed here may be due to direct transmission, although further analysis is required to confirm this.

Porphyromonas gingivalis and *A. actinomycetemcomitans* showed a high odds ratio among the periodontopathic bacteria investigated. These two microorganisms have fimbriae as structures for adherence.[33,34] Fimbriae-deficient mutants of both species were reported to show reduced virulence.[35,36] This property may have been the reason for the high odds ratio seen here. The weakest correlation in colonization between adjacent teeth and implants was observed for *F. nucleatum*. This microorganism was reported to attach to a large number of species and acts as a bridge between early colonizers such as streptococci and late colonizers such as *Porphyromonas gingivalis*.[37] *F. nucleatum* was detected not only in adults, but also in children.[25] This fastidious microorganism also showed the highest prevalence in the present study. This may be why the correlation between the species tested with implants and adjacent teeth was relatively low. No correlation was observed between detection of *Tannerella forsythia* in peri-implant sulci and in the gingival crevices of natural

TABLE 3 Multiple Logistic Regression Analysis (Stepwise Method) of Risk of Transmission of Periodontopathic Bacteria from Adjacent Natural Teeth to Implants		
Bacteria	Odds Ratio (95% CI)*	*p*-Value
Aggregatibacter actinomycetemcomitans	7.281 (1.39–38.20)	0.0199
Prevotella intermedia	4.917 (1.50–16.08)	0.0084
Porphyromonas gingivalis	7.516 (1.72–32.90)	0.0074
Treponema denticola	4.884 (1.27–18.72)	0.0207
Tannerella forsythia	–	–
Fusobacterium nucleatum	3.575 (1.03–12.44)	0.0453

*95% confidence interval.

teeth. Some bacteria also produce bacteriocins to compete with other microorganisms. It is possible that bacteriocins were produced following interactions among other biofilm constituents, thus inhibiting colonization by this microorganism.

Taken together, these results suggest that the adjacent teeth strongly affect colonization by periodontopathic bacteria in the peri-implant sulcus. This indicates the importance of elimination of periodontopathic bacteria from adjacent teeth prior to implant treatment in preventing subsequent failure because of peri-implantitis.

ACKNOWLEDGMENTS

REFERENCES

1. Stanford CM. Dental implants. A role in geriatric dentistry for the general practice? J Am Dent Assoc 2007; 138(Suppl):34S–40S.

2. Grossmann Y, Nissan J, Levin L. Clinical effectiveness of implant-supported removable partial dentures: a review of the literature and retrospective case evaluation. J Oral Maxillofac Surg 2009; 67:1941–1946.

3. Pjetursson BE, Lang NP. Prosthetic treatment planning on the basis of scientific evidence. J Oral Rehabil 2008; 35(Suppl 1):72–79.

4. Kourtis SG, Sotiriadou S, Voliotis S, Challas A. Private practice results of dental implants. Part I: survival and evaluation of risk factors – Part II: surgical and prosthetic complications. Implant Dent 2004; 13:373–385.

5. Albrektsson T, Isidor F. Consensus report of session IV. In: Lang NP, Karring TS, eds. Proceedings of the 1st European Workshop on Periodontolog. London: Quintessence Publishing Co. Ltd., 1994:365–369.

6. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005; 366:1809–1820.

7. Lang NP, Wilson TG, Corbet EF. Biological complications with dental implants: their prevention, diagnosis and treatment. Clin Oral Implants Res 2000; 11(Suppl 1):146–155.

8. Mombelli A, Lang NP. The diagnosis and treatment of peri-implantitis. Periodontol 2000 1998; 17:63–76.

9. Heitz-Mayfield LJ. Peri-implant diseases: diagnosis and risk indicators. J Clin Periodontol 2008; 35:292–304.

10. Apse P, Ellen RP, Overall CM, Zarb GA. Microbiota and crevicular fluid collagenase activity in the osseointegrated dental implant sulcus: a comparison of sites in edentulous and partially edentulous patients. J Periodontal Res 1989; 24:96–105.

11. Papaioannou W, Bollen CM, Van Eldere J, Quirynen M. The adherence of periodontopathogens to periodontal probes. A possible factor in intra-oral transmission? J Periodontol 1996; 67:1164–1169.

12. Mombelli A, van Oosten MA, Schurch E Jr, Land NP. The microbiota associated with successful or failing osseointegrated titanium implants. Oral Microbiol Immunol 1987; 2:145–151.

13. Leonhardt A, Renvert S, Dahlen G. Microbial findings at failing implants. Clin Oral Implants Res 1999; 10:339–345.

14. Augthun M, Conrads G. Microbial findings of deep peri-implant bone defects. Int J Oral Maxillofac Implants 1997; 12:106–112.

15. Hultin M, Gustafsson A, Klinge B. Long-term evaluation of osseointegrated dental implants in the treatment of partly edentulous patients. J Clin Periodontol 2000; 27:128–133.

16. Karoussis IK, Salvi GE, Heitz-Mayfield LJ, Bragger U, Hammerle CH, Lang NP. Long-term implant prognosis in patients with and without a history of chronic periodontitis: a 10-year prospective cohort study of the ITI Dental Implant System. Clin Oral Implants Res 2003; 14:329–339.

17. Hardt CR, Grondahl K, Lekholm U, Wennstrom JL. Outcome of implant therapy in relation to experienced loss of periodontal bone support: a retrospective 5-year study. Clin Oral Implants Res 2002; 13:488–494.

18. Takanashi K, Kishi M, Okuda K, Ishihara K. Colonization by *Porphyromonas gingivalis* and *Prevotella intermedia* from teeth to osseointegrated implant regions. Bull Tokyo Dent Coll 2004; 45:77–85.

19. Mousques T, Listgarten MA, Phillips RW. Effect of scaling and root planing on the composition of the human subgingival microbial flora. J Periodont Res 1980; 15:144–151.

20. Magnusson I, Lindhe J, Yoneyama T, Liljenberg B. Recolonization of a subgingival microbiota following scaling in deep pockets. J Clin Periodontol 1984; 11:193–207.

21. van Winkelhoff AJ, van der Velden U, de Graaff J. Microbial succession in recolonizing deep periodontal pockets after a single course of supra- and subgingival debridement. J Clin Periodontol 1988; 15:116–122.

22. Wade WG, Moran J, Morgan JR, Newcombe R, Addy M. The effects of antimicrobial acrylic strips on the subgingival microflora in chronic periodontitis. J Clin Periodontol 1992; 19:127–134.

23. Sumida S, Ishihara K, Kishi M, Okuda K. Transmission of periodontal disease-associated bacteria from teeth to osseointegrated implant regions. Int J Oral Maxillofac Implants 2002; 17:696–702.

24. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol Immunol 1996; 11:266–273.

25. Kobayashi N, Ishihara K, Sugihara N, Kusumoto M, Yakush-iji M, Okuda K. Colonization pattern of periodontal bacteria in Japanese children and their mothers. J Periodontal Res 2008; 43:156–161.

26. Mombelli A, Lang NP. Antimicrobial treatment of peri-implant infections. Clin Oral Implants Res 1992; 3:162–168.

27. Rosenberg ES, Torosian JP, Slots J. Microbial differences in 2 clinically distinct types of failures of osseointegrated implants. Clin Oral Implants Res 1991; 2:135–144.

28. Amano A, Sharma A, Lee JY, Sojar HT, Raj PA, Genco RJ. Structural domains of *Porphyromonas gingivalis* recombinant fimbrillin that mediate binding to salivary proline-rich protein and statherin. Infect Immun 1996; 64:1631–1637.

29. Onagawa M, Ishihara K, Okuda K. Coaggregation between *Porphyromonas gingivalis* and Treponema denticola. Bull Tokyo Dent Coll 1994; 35:171–181.

30. Kolenbrander PE, Andersin RN, Moore LVH. Coaggregation of *Fusobacterium nucleatum, Selenomonas flueggei, Selenomonas inferix, Selenomonas noxia*, and *Selenomonas sputigena* with strains from 11 genera of oral bacteria. Infect Immun 1989; 57:3194–3203.

31. Ikegami A, Honma K, Sharma A, Kuramitsu HK. Multiple functions of the leucine-rich repeat protein LrrA of *Treponema denticola*. Infect Immun 2004; 72:4619–4627.

32. Kremer BH, van Steenbergen TJ. *Peptostreptococcus micros* coaggregates with *Fusobacterium nucleatum* and non-encapsulated Porphyromonas gingivalis. FEMS Microbiol Lett 2000; 182:57–62.

33. Inouye T, Ohta H, Kokeguchi S, Fukui K, Kato K. Colonial variation and fimbriation of *Actinobacillus actinomycetemcomitans*. FEMS Microbiol Lett 1990; 57:13–17.

34. Yoshimura F, Takahashi K, Nodasaka Y, Suzuki T. Purification and characterization of a novel type of fimbriae from the oral anaerobe *Bacteroides gingivalis*. J Bacteriol 1984; 160:949–957.

35. Schreiner HC, Sinatra K, Kaplan JB, et al. Tight-adherence genes of *Actinobacillus actinomycetemcomitans* are required for virulence in a rat model. Proc Natl Acad Sci U S A 2003; 100:7295–7300.

36. Malek R, Fisher JG, Caleca A, et al. Inactivation of the *Porphyromonas gingivalis fimA* gene blocks periodontal damage in gnotobiotic rats. J Bacteriol 1994; 176:1052–1059.

37. Kolenbrander PE, London J. Adhere today, here tomorrow: oral bacterial adherence. J Bacteriol 1993; 175:3247–3252.