Transmission of Periodontal Disease–Associated Bacteria from Teeth to Osseointegrated Implant Regions

Shinichi Sumida, PhD, DDS[1]/Kazuyuki Ishihara, PhD, DDS[2]/Masataka Kishi, PhD, DDS[3]/
Katsuji Okuda, PhD, DDS[4]

Purpose: *The presence of periodontopathic bacteria is a risk factor for peri-implantitis. The present study examined colonization by periodontopathic bacteria and their transmission from periodontal pockets to osseointegrated implant sulcus.* ***Materials and Methods:*** *Plaque samples were collected from 105 sites in the 15 patients who participated in the study. Colonization by these bacteria was examined by polymerase chain reaction (PCR) and culture. The transmission of periodontopathic bacteria from periodontal sites of natural teeth to the implant sulcus was analyzed by pulsed field gel electrophoresis (PFGE).* ***Results:*** *The PCR detection rates of Porphyromonas gingivalis, Prevotella intermedia, Actinobacillus actinomycetemcomitans, Bacteroides forsythus, and Treponema denticola were 80.0%, 53.3%, 46.7%, 60.0% and 40.0%, respectively. Colonizations by P gingivalis and A actinomycetemcomitans were statistically correlated with periodontal pockets and implant sulcus regions (P < .01). The PFGE patterns of the P gingivalis strains isolated from each patient were identical, but differed from those from other patients. The PFGE patterns of P intermedia strains were identical in 2 out of 3 patients.* ***Discussion:*** *These analyses indicated that there appeared to be transmission of P gingivalis and P intermedia from the periodontal pocket to the peri-implant region.* ***Conclusion:*** *Elimination of these periodontal pathogens from the patient's oral cavity before administering dental implant treatment may inhibit colonization by these pathogens and reduce the risk of peri-implantitis.* (INT J ORAL MAXILLOFAC IMPLANTS 2002;17:696–702)

Key words: *disease transmission, peri-implantitis, periodontopathic bacterial infection*

COPYRIGHT © 2002 BY QUINTESSENCE PUBLISHING CO, INC. PRINTING OF THIS DOCUMENT IS RESTRICTED TO PERSONAL USE ONLY. NO PART OF THIS ARTICLE MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM WITHOUT WRITTEN PERMISSION FROM THE PUBLISHER.

Osseointegrated implants have frequently been used to support denture prostheses in edentulous patients, and favorable results have been reported.[1–7] However, one possible outcome of unsuccessful treatment is peri-implantitis. Over 300 species of bacteria are present in the oral cavity, each region of which is home to a characteristic assemblage of microflora.[8] In the periodontal pockets of adult periodontitis patients, anaerobic Gram-negative rods proliferate as the periodontal disease progresses.[9] Of these bacteria, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, and *Treponema denticola* play important roles in the advancement of this disease. Over the past several years, the microflora active in implantitis have been reported to be similar in composition to those that cause periodontitis.[10–16] In addition, staphylococci are also frequently detected around implants.[17] The pathogens of implantitis are considered to propagate from the periodontopathic bacteria of natural teeth to the vicinity of implants. Even in edentulous

[1]Researcher, Department of Removable Partial Prosthodontics, Tokyo Dental College, Tokyo, Japan.
[2]Associate Professor, Department of Microbiology, Tokyo Dental College, Tokyo, Japan.
[3]Professor, Department of Removable Partial Prosthodontics, Tokyo Dental College, Tokyo, Japan.
[4]Professor and Chairman, Department of Microbiology, Tokyo Dental College, Tokyo, Japan.

Reprint requests: Dr Katsuji Okuda, Department of Microbiology, Tokyo Dental College 1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan. Fax: +81-43-270-3744. E-mail: kokuda@tdc.ac.jp

COPYRIGHT © 2002 BY QUINTESSENCE PUBLISHING CO, INC. PRINTING OF THIS DOCUMENT IS RESTRICTED TO PERSONAL USE ONLY. NO PART OF THIS ARTICLE MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM WITHOUT WRITTEN PERMISSION FROM THE PUBLISHER.

Table 1 Dental Implant Patient Data

Patient no.	Gender	Age (y)	Years since operation	Location and no. of implants applied
1	F	47	10	MNR 2, MNL 2
2	F	56	3	MNL 2
3	M	42	11	MNL 3
4	F	63	1	MXL 2, MNR 3
5	M	21	1	MNL 4
6	F	66	5	MNR 3, MNL 3
7	M	44	1	MNL 3
8	F	59	1	MXR 3, MXM 1, MXL 1, MNL 3
9	M	62	7	MXR 4, MXM 1, MNL 4
10	F	50	2	MNR 3, MXL 3
11	F	65	6	MXM 1, MXL 1, MNR 1, MNL 1
12	F	49	1	MNR 3, MNL 4,
13	M	63	3	MXR 1, MXL 1, MNR 1, MNL 1
14	F	56	7	MNR 4, MNL 2
15	F	55	1	MNL 3

MXR = Maxillary right sextant; MXM = maxillary mid sextant; MXL = maxillary left sextant; MNR = mandibular right sextant; MNL = mandibular left sextant.

patients with histories of periodontitis, *P gingivalis* and *A actinomycetemcomitans* were not detected in sites of peri-implantitis; the microflora in the gingival sulci resembled those seen in healthy periodontal tissues and in periodontitis sites.[18]

In the present study, colonization by periodontal disease–associated bacteria was examined, and black-pigmented anaerobic rods were isolated from gingival crevices or periodontal pockets of natural teeth in regions with endosseous implant sulci. The data were analyzed to ascertain whether or not transmission from the periodontal pockets of natural teeth to the peri-implant tissues had taken place.

MATERIALS AND METHODS

Subjects

The subjects of this study were 15 patients (10 women, 5 men) who visited the Department of Prosthodontics, Tokyo Dental College, Chiba, Japan, and received endosseous implants in partially edentulous regions. Informed consent was obtained from all subjects in the study. Their clinical data are presented in Table 1. The ages of the patients ranged from 21 to 66 years (mean 51.2 ± 11.9 years), and the loss of teeth at the sites for implant placement was the result of periodontitis in all cases. The intervals after these patients received implants and had superstructures fitted ranged from 1 year to 11 years (mean 4 years). It was verified that they had not taken any antibiotics for 6 months before sampling was performed. Clinical examinations, including probing depth (PD, in mm) and the presence of bleeding on probing (BOP) using a WHO-type pocket probe, were performed.

Sampling of Subgingival Plaque

Briefly, supragingival plaque was removed, and the regions of the teeth to be examined were dried gently with sterile cotton rolls. The subgingival plaque was collected with a sterilized plastic scaler (Nobel Biocare, Göteborg, Sweden) from both natural teeth and implants. The obtained plaque sample in each case was transferred to 100 µL of reduced transport fluid (RTF),[19] and the sample was dispersed by mixing with 0.8-mm-diameter glass beads using a Vortex mixer for 15 seconds.[20] A part of each suspension was cultured. The microorganisms were sedimented by centrifugation at 15,000×*g* at 4°C for 10 minutes.

COPYRIGHT © 2002 BY QUINTESSENCE PUBLISHING CO, INC. PRINTING OF THIS DOCUMENT IS RESTRICTED TO PERSONAL USE ONLY. NO PART OF THIS ARTICLE MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM WITHOUT WRITTEN PERMISSION FROM THE PUBLISHER.

Table 2 List of Primers Used

Species	Sequence	Product size
P gingivalis	5′-ATA ATG GAG AAC AGC AGG AA-3′ 5′-TCT TGC CAA CCA GTT CCA TTG C-3′	131 (bp)
A actinomycetemcomitans	5′-CAG CAA GCT GCA CAG TTT GCA AA-3′ 5′-CAT TAG TTA ATG CCG GGC CGT CT-3′	238 (bp)
B forsythus	5′-GCG TAT GTA ACC TGC CCG CA-3′ 5′-TGC TTC AGT GTC AGT TAT ACC T-3′	641 (bp)
T denticola	5′-TAA TAC CGA ATG TGC TCA TTT ACA T-3′ 5′-TCA AAG AAG CAT TCC CTC TTC TTC TTA-3′	316 (bp)
P intermedia	5′-TTT GTT GGG GAG TAA AGC GGG-3′ 5′-TCA ACA TCT CTG TAT CCT GCG T-3′	575 (bp)

Detection of Pathogens with Polymerase Chain Reaction

Obtained samples were examined for the presence of *P gingivalis*, *A actinomycetemcomitans*, *B forsythus*, *T denticola*, and *P intermedia* by the polymerase chain reaction (PCR), as described by Watanabe and Frommel[21,22] and Ashimoto and coworkers.[23] Sedimented microorganisms were suspended in 50 µL of boiling buffer (20 mmol/L Tris-HCl pH 8.0, 2 mmol/L EDTA, 1% triton X-100) and boiled at 100°C for 10 minutes. After the removal of cell debris by centrifugation at 15,000×*g* at 4°C for 15 minutes, the supernatant was extracted with phenol and precipitated by ethanol. The specific primer pairs used in the PCR are summarized in Table 2. Briefly, 5 µL of sample was added to 45 µL of the reaction mixture, which was composed of PCR buffer (Takara, Shiga, Japan) containing 0.2 mmol/L dNTP, the specific primer pairs listed in Table 2, and 0.25 U Taq DNA polymerase (Takara). The PCR assays for *P gingivalis* and *A actinomycetemcomitans* were performed using a thermal cycler (Gene Amp PCR system 9700, PE Biosystems, Foster City, CA) under the following conditions: 36 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension step at 72°C for 1 minute. The PCR assays for *B forsythus*, *T denticola*, and *P intermedia* were performed using a thermal cycler as follows: 36 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 1 minute, and extension step at 72°C for 1 minute. The PCR products were electrophoresced on 2% agarose gel and examined under ultraviolet light following staining with ethidium bromide.

Analysis by Pulsed Field Gel Electrophoresis

A part of each plaque sample was diluted with RTF solution in a gradient of 1:10 steps down to $1{:}10^{-5}$, and 100 µL of each dilution was inoculated onto trypticase soy agar (BBL, Cockysville, MD) containing 5 µg/mL hemin, 0.5 µg/mL menadione, and 10% horse defibrinated blood. The plates were cultured in an anaerobic chamber containing 10% CO_2, 10% H_2, and 80% N_2 at 37°C for 5 to 7 days. Black-pigmented colonies were re-inoculated on blood agar plates for isolation. All isolated strains were identified by sequences of 16s rRNA locus. From these colonies, *P gingivalis* and *P intermedia* were subjected to pulsed field gel electrophoretic (PFGE) analysis. Single colonies of *P gingivalis* and *P intermedia* taken from each patient were inoculated into trypticase soy broth (BBL) containing 5 µg/mL of hemin and 0.5 µg/mL of menadione and cultured for 2 days. The bacterial cells were then harvested by centrifugation at 15,000×*g* at 4°C for 15 minutes.

The cells obtained were treated using the method described by Nakayama.[24] Briefly, after all were embedded in agarose gel blocks, cells were lysed with a solution containing 1.0% SDS, proteinase K (2 mg/mL), and 1 mmol/L EDTA. After washing of the block, genomic DNA was digested with *Not* I, *Spe* I, or *Sma* I. Using a 1% agarose gel, electrophoresis with a CHEF-DR III apparatus (Biorad, Hercules, CA) was performed on the resulting material under the following conditions: potential difference, 6 volts/cm; electrode angle, 120 degrees; initial switch time, 5.3 seconds; final switch time, 49.9 seconds; and total duration, 20 hours. After completion of the electrophoresis, the gel was placed in 1.2 µg/mL ethidium bromide for 60 minutes, and, after being stained, was photographed under ultraviolet light.

Statistical Analysis

The relationships between the colonizations by the 5 bacterial species from natural teeth and implant regions were analyzed by the chi-squared test.

COPYRIGHT © 2002 BY QUINTESSENCE PUBLISHING CO, INC. PRINTING OF THIS DOCUMENT IS RESTRICTED TO PERSONAL USE ONLY. NO PART OF THIS ARTICLE MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM WITHOUT WRITTEN PERMISSION FROM THE PUBLISHER.

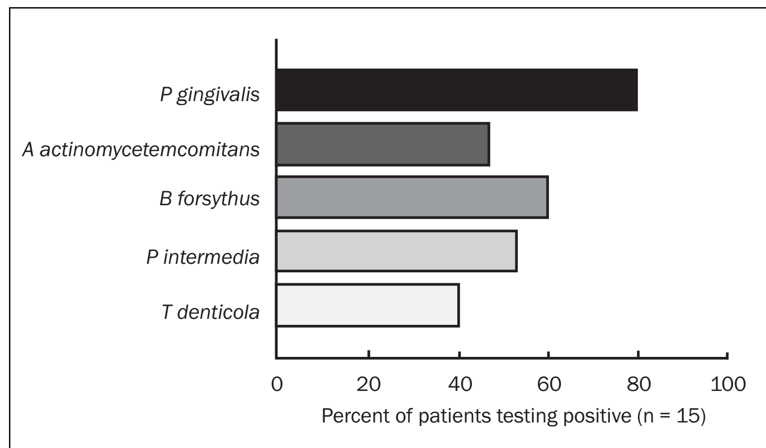

Fig 1 Rates of periodontopathogen detection by PCR in 15 patients.

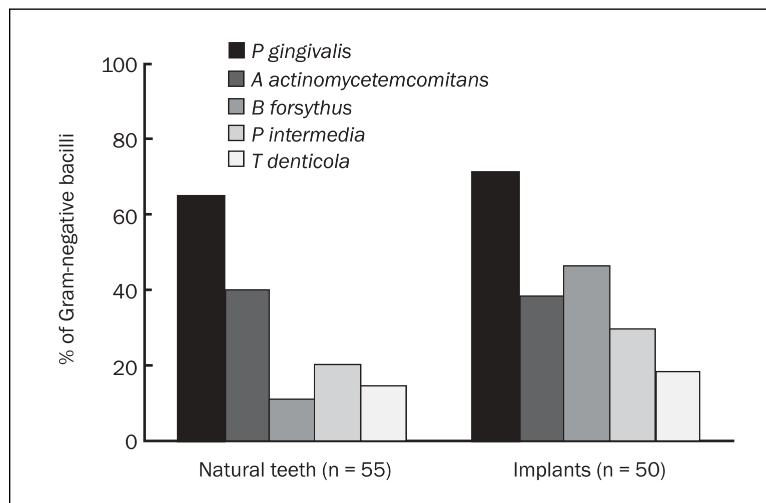

Fig 2 Rates of periodontopathogen detection by PCR in experimental sites.

RESULTS

Clinical Data

The PDs at the sites from which the samples were collected were between 4 and 7 mm (mean 4.6 ± 0.8 mm) at the periodontal pockets and between 4 and 5 mm (mean 4.1 ± 0.4 mm) at the implant sites. BOP was negative in all cases. Thus it was assumed that there was no disease clinically present in tissues around the implants.

Detection Rates of Periodontopathogens with PCR

A total of 105 samples isolated from gingival crevices of implants (n = 50) and natural teeth (n = 55) were examined for detection of periodontopathic bacteria. As Fig 1 shows, the detected proportions of *P gingivalis*, *A actinomycetemcomitans*, *B forsythus*, *P intermedia*, and *T denticola* by PCR in the 15 subjects in this study were 80.0%, 46.7%, 60.0%, 53.3%, and 40.0%, respectively. There was no difference in detection because of gender, probing depth, or time since last cleaning.

Comparison of the proportion of Gram-negative bacilli in 55 periodontal pockets around natural teeth and in 50 such sites around implants is illustrated in Fig 2. *P gingivalis*, *A actinomycetemcomitans*, and *T denticola* were detected in proportions of 65.5% and 72.0%, 40.0% and 38.0%, and 14.5% and 18.0% at teeth and implant regions, respectively; the rates in the 2 types of sites were very similar. For *B forsythus* and *P intermedia*, however, rates were 10.9% and 46.0% and 20.0% and 30.0% at teeth and implant regions, respectively, showing that Gram-negative rods were detected at higher frequencies around implants than in periodontal pockets.

Detected proportions of the 5 examined species, including mixed colonizations, are summarized in Table 3. In natural teeth and implant regions, sites without detection of any bacterial species were 25.6% in natural teeth and 14.0% in implant regions, respectively. Two or more bacterial species

COPYRIGHT © 2002 BY QUINTESSENCE PUBLISHING CO, INC. PRINTING OF THIS DOCUMENT IS RESTRICTED TO PERSONAL USE ONLY. NO PART OF THIS ARTICLE MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM WITHOUT WRITTEN PERMISSION FROM THE PUBLISHER.

Table 3 Percentage of Mixed Infection by Periodontopathic Bacterial Species Among All Sites Sampled

No. of species	Natural teeth (positive % in 55 sites)	Implants (positive % in 50 sites)
5	1.8	2.0
4	3.6	16.0
3	14.5	24.0
2	25.5	14.0
1	29.0	30.0
No species detected	25.6	14.0

Table 4 Periodontopathogen Detection Around the Implants

	Detection from implant (%)	
Species	Patients with natural teeth positive	Patients with natural teeth negative
P gingivalis	11/12 (91.7)	3/3 (100)*
A actinomycetemcomitans	7/7 (100)	8/8 (100)*
B forsythus	2/2 (100)	6/13 (46.7)
T denticola	3/4 (75.0)	9/11 (81.8)
P intermedia	4/5 (80.0)	7/10 (70.0)

* Statistically significant difference at $P < .01$.

were detected in 45.4% of samples from natural teeth and 56.0% of those from implant sites. The relationships between the 5 bacteria detected in natural teeth and implant regions were examined (Table 4). Detection of *P gingivalis* and *A actinomycetemcomitans* at natural teeth was related to that at implants in all 15 patients ($P < .01$). No statistical correlations were found for *B forsythus*, *P intermedia*, or *T denticola* in these patients.

PFGE

It was possible to isolate *P gingivalis* and *P intermedia* strains from both natural teeth and peri-implant sulcus regions from 4 and 3 patients, respectively. There were no corresponding PFGE patterns in *P gingivalis* strains isolated from different patients. The patterns of *P intermedia* strains from different patients were also dissimilar. However, the patterns of PFGE of *P gingivalis* strains isolated from each patient were identical.

The PFGE results from *P gingivalis* strains from patients No. 1, No. 4, No. 8, and No. 12 are presented in Fig 3. There were no clearly different electrophoresis patterns between strains isolated from the sites of natural teeth (N) and those of implants (I) from the same patient when digested with *Not* I. To confirm that these 2 strains were identical, sample No.1 was digested with *Spe* I, and it was found that the PFGE patterns again matched.

The patterns of *P intermedia* strains isolated from patients No. 1, No. 4, and No. 8 are shown in Fig 4. No corresponding PFGE patterns of *P intermedia* isolates from different patients were found to be identical. In patients No. 4 and No. 8, the patterns of the strains from implant sites and natural teeth were identical in each patient, but the patterns of the strains isolated from implant sites and natural teeth in patient No. 1 were different.

DISCUSSION

In the past decade, studies of the microbial flora found at implant sites have been reported by many groups.[25–28] In the tissue surrounding titanium implants, formation of dental plaque can be observed within 24 hours. The rough nature of the surface of implants has been reported to encourage colony formation by the bacteria,[28] so efforts are being made to develop materials that can inhibit colonization by periodontopathogens. Ashimoto and associates[23] reported that the proportions of PCR detection of *P gingivalis*, *P intermedia*, *A actinomycetemcomitans*, *B forsythus*, and *T denticola* in subgingival plaque in advanced periodontitis lesions were 73%, 58%, 30%, 85%, and 54%, respectively. The present study showed that the detected proportions in samples obtained from implant regions

COPYRIGHT © 2002 BY QUINTESSENCE PUBLISHING CO, INC. PRINTING OF THIS DOCUMENT IS RESTRICTED TO PERSONAL USE ONLY. NO PART OF THIS ARTICLE MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM WITHOUT WRITTEN PERMISSION FROM THE PUBLISHER.

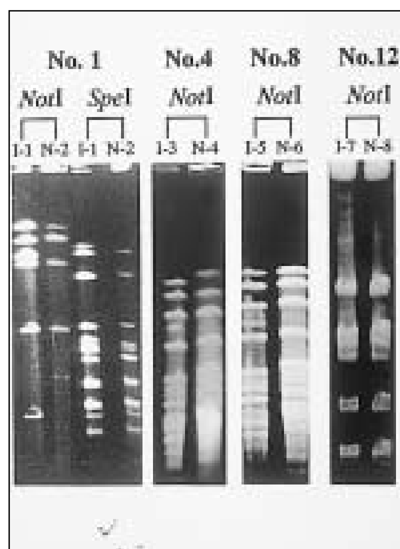

Fig 3 *(Left)* PFGE of *P gingivalis* isolated from patients No. 1, No. 4, No. 8, and No. 12 and digested with *Not* I or *Spe* I. Identical patterns of strains isolated from implant sites and natural teeth from the same patients were seen, but differing patterns were noted between strains isolated from different patients. I = strain isolated from implant region; N = strain isolated from natural teeth.

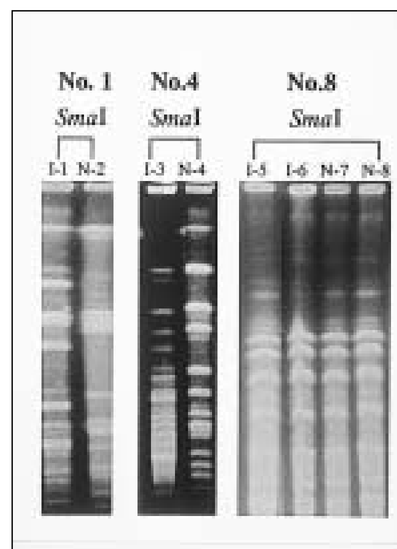

Fig 4 *(Right)* PFGE of *P intermedia* isolated from patients No. 1, No. 4, and No. 8 and digested with *Sma* I. Identical patterns of matched strains from implant sites and natural teeth isolated from patients No. 4 and No. 8 were seen, but the patterns of the 2 strains isolated from patient No. 1 differed. I = strain isolated from implant region; N = strain isolated from natural teeth.

were similar to those previously reported. Kalykakis and coworkers[17] and Leonhardt and colleagues[25] examined detection rates of periodontal pathogens by culture studies from implant sulci. In those studies, the proportions detected were lower than those in the present study. To clarify the periodontal status and colonization by periodontopathic bacteria at implants, more subjects, including patients with peri-implantitis, and more studies using both culture and PCR methods are required in the future.

Analysis of the PFGE patterns of isolated *P gingivalis* and *P intermedia* strains revealed differences within individual subjects. There were 4 subjects in whom both *P gingivalis* strains were isolated from both natural teeth and implants; each pair of implant and natural tooth samples from the same patient showed the same digestion pattern. This fact suggests that these organisms had migrated from the natural teeth to the implant areas. The PFGE of the pairs of *P intermedia* strains isolated from 2 patients showed the strains to be identical, but the pair of samples from a third patient were not. Umeda and associates[29] reported the detection of periodontopathogens such as *P gingivalis* in saliva, and Amano and coworkers[30] stated that *P gingivalis* could adhere to statherin, a salivary component. They suggested that these characteristics can play an important role in colonization. The above studies suggest that *P gingivalis* can migrate from the periodontal pocket into the saliva and thus be transmitted to the vicinity of an implant. Akagawa and colleagues[31] demonstrated that 3 months of plaque control kept the microbial flora in implant sites similar to the normal pattern. This report suggested a need for plaque control in the implant sulcus.

SUMMARY

The present study supported the hypothesis that some periodontopathogens colonizing implant sites can be transmitted from the natural teeth. Consequently, elimination of these periodontal pathogens from the patient's oral cavity before administering dental implant treatment may inhibit colonization by these pathogens and reduce the risk of peri-implantitis.

REFERENCES

1. Adell R, Lekholm U, Rockler B, Brånemark P-I. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. Int J Oral Surg 1981;10: 387–416.
2. Jemt T, Lekholm U, Adell R. Osseointegrated implants in the treatment of partially edentulous patients: A preliminary study on 876 consecutively placed fixtures. Int J Oral Maxillofac Implants 1989;4:211–217.
3. Tzakis MG, Linden B, Jemt T. Oral function in patients treated with prostheses on Brånemark osseointegrated implants in partially edentulous jaws: A pilot study. Int J Oral Maxillofac Implants 1990;5:107–111.
4. Jemt T, Lekholm U. Oral implant treatment in posterior partially edentulous jaws: A 5-year follow-up report. Int J Oral Maxillofac Implants 1993;8:635–640.
5. Lekholm U, van Steenberghe D, Herrmann I, et al. Osseointegrated implants in the treatment of partially edentulous jaws: A prospective 5-year multicenter study. Int J Oral Maxillofac Implants 1994;9:627–635.
6. Lekholm U, Gunne J, Henry P, et al. Survival of the Brånemark implant in partially edentulous jaws: A 10-year prospective multicenter study. Int J Oral Maxillofac Implants 1999;14:639–645.

COPYRIGHT © 2002 BY QUINTESSENCE PUBLISHING CO, INC. PRINTING OF THIS DOCUMENT IS RESTRICTED TO PERSONAL USE ONLY. NO PART OF THIS ARTICLE MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM WITHOUT WRITTEN PERMISSION FROM THE PUBLISHER.

7. Noack N, Willer J, Hoffmann J. Long-term results after placement of dental implants: Longintudinal study of 1,964 implants over 16 years. Int J Oral Maxillofac Implants 1999;14:748–755.
8. Paster BJ, Boches SK, Galvin JL, et al. Bacterial diversity in human subgingival plaque. J Bacteriol 2001;183:3770–3783.
9. Moore WEC, Holdeman LV, Cato EP, Smibert RM, Burmeister JA, Ranney RR. Bacteriology of moderate (chronic) periodontitis in mature adult humans. Infect Immun 1983;42:510–515.
10. Dharmar S, Yoshida K, Adachi Y, Kishi M, Okuda K, Sekine H. Subgingival microbial flora associated with Brånemark implants. Int J Oral Maxillofac Implants 1994;9:314–318.
11. Silverstein LH, Kurtzman D, Garnick JJ, Schuster GS, Steflik DE, Moskowitz ME. The microbiota of the peri-implant region in health and disease. Implant Dent 1994;3:170–174.
12. Eke PI, Braswell LD, Fritz ME. Microbiota associated with experimental peri-implantitis and periodontitis in adult *Macaca mulatta* monkeys. J Periodontol 1998;69:190–194.
13. Ellen RP. Microbial colonization of the peri-implant environment and its relevance to long-term success of osseointegrated implants. Int J Prosthodont 1998;11:433–441.
14. Mombelli A, Feloutzis A, Bragger U, Lang NP. Treatment of peri-implantitis by local delivery of tetracycline. Clinical, microbiological and radiological results. Clin Oral Implants Res 2001;12:287–294.
15. Mombelli A. Microbiology and antimicrobial therapy of peri-implantitis. Periodontol 2000 2002;28:177–189.
16. Quirynen M, De Soete M, van Steenberghe D. Infectious risks for oral implants: A review of the literature. Clin Oral Implants Res 2002;13:1–19.
17. Kalykakis G, Zafiropoulos GG, Murat Y, Hubertus S, Nisengard RJ. Clinical and microbiological status of osseointegrated implants. J Periodontol 1994;65:766–770.
18. Danser MM, van Winkelhoff AJ, van der Velden U. Periodontal bacteria colonizing oral mucous membranes in edentulous patients wearing dental implants. J Periodontol 1997; 68:209–216.
19. Syed SA, Loesche WJ. Survival of human dental plaque flora in various transport media. Appl Microbiol 1972;24: 638–644.
20. Ishihara K, Miura T, Kimizuka R, Okuda K, Ebihara Y, Mizuno Y. Oral bacteria inhibit *Helicobacter pylori* growth. FEMS Microbiol Lett 1997;152:355–361.
21. Watanabe K, Frommel TO. Detection of *Porphyromonas gingivalis* in oral plaque samples by use of the polymerase chain reaction. J Dent Res 1993;72:1040–1044.
22. Watanabe K, Frommel TO. *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* and *Treponema denticola* detection in oral plaque samples using the polymerase chain reaction. J Clin Periodontol 1996;23:212–219.
23. 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.
24. Nakayama K. Determination of the genome size of the oral anaerobic bacterium *Porphyromonas gingivalis* by pulsed field gel electrophoresis. Dent Jpn 1995;32:25–28.
25. Leonhardt A, Berglundh T, Ericsson I, Dahlen G. Putative periodontal pathogens on titanium implants and teeth in experimental gingivitis and periodontitis in beagle dogs. Clin Oral Implants Res 1992;3:112–119.
26. Lindhe J, Berglundh T, Ericsson I, Liljenberg B, Marinello C. Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. Clin Oral Implants Res 1992;3:9–16.
27. Rimondini L, Fare S, Brambilla E, et al. The effect of surface roughness on early in vivo plaque colonization on titanium. J Periodontol 1997;68:556–562.
28. Keller W, Bragger U, Mombelli A. Peri-implant microflora of implants with cemented and screw-retained suprastructures. Clin Oral Implants Res 1998;9:209–217.
29. Umeda M, Contreras A, Chen C, Bakker I, Slots J. The utility of whole saliva to detect the oral presence of periodontopathic bacteria. J Periodontol 1998;69:828–833.
30. 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.
31. Akagawa Y, Matsumoto T, Kawamura M, Tsuru H. Changes of subgingival microflora around single-crystal sapphire endosseous implants after experimental ligature-induced plaque accumulation in monkeys. J Prosthet Dent 1993;69:594–598.