

DNA Probe Identification of Bacteria Colonizing Internal Surfaces of the Implant-Abutment Interface: A Preliminary Study

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Background: Currently, there is limited knowledge concerning the specific genus and species of bacteria that may colonize internal surfaces of the implant-abutment interface (IAI) of two-stage dental implants. The purpose of this study was to use DNA probe analysis to identify those periodontopathic bacteria that may inhabit the internal surfaces and healing abutment screw-threads of the IAI of dental implants *in situ*.

Methods: Following osseointegration, bacterial samples for DNA probe analysis were obtained from 54 two-stage hydroxyapatite plasma spray-coated implants in 32 patients. Using sterile paper points, samples were obtained from the IAI of 43 implants and the screw-threads of healing abutments in the other 11 implants. DNA probes were available to detect the following microbes: *Actinobacillus actinomycetemcomitans*, *Tannerella forsythensis*, *Campylobacter rectus*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Treponema denticola*.

Results: All samples taken from healing abutment screw-threads were negative for all target microbes. The aggregate percentage of positive results for each target microbe for samples from internal surfaces of the IAI were: 41.9%, *A. actinomycetemcomitans*; 60.5%, *T. forsythensis*; 44.2%, *C. rectus*; 60.5%, *E. corrodens*; 48.8%, *F. nucleatum*; 46.5%, *P. gingivalis*; 55.8%, *P. intermedia*; and 51.2%, *T. denticola*. In addition, no significant differences were noted between colonization of individual microbial species when comparing anterior to posterior and maxillary to mandibular implant sites.

Conclusions: Moderate to high levels of eight different periodontopathic microbes inhabiting the internal surfaces of the IAI of 43 two-stage implants in partially edentulous patients were identified by DNA probe analysis. The microbes colonized these surfaces within 25 days following the second stage surgery and placement of the healing abutment. In contrast, all samples obtained from screw-threads of 11 healing abutments were DNA probe negative. These findings appear to support those of other investigations demonstrating the translocation of bacteria from residual dentition to implants. *J Periodontol* 2005;76:115-120.

KEY WORDS

Dental implants/microbiology; DNA probes; peri-implant diseases/microbiology.

The physical design of several dental implant systems requires a two-stage surgery prior to final restoration. The two-stage design dictates insertion of the implant body at or below (countersinking) the bony alveolar crest. The rationale for this technique takes into consideration several factors: prevention of implant interface movement during bone healing and remodeling; prevention of implant exposure during healing; and enhancement of emergence profile for implant restorations at the expense of crestal bone. However, once the prosthetic abutment is positioned, a microgap at the implant-abutment interface (IAI) is created that lies in close proximity to or just below the bony crest. The presence of the IAI microgap in close approximation to bone is considered by several investigators to have a major role in peri-implant inflammation and circumferential bone loss.¹⁻⁵

Although the actual significance of the IAI microgap and its location is somewhat conjectural,^{1,5,6} studies using canine experimental models indicate that as much as 2 mm of crestal bone loss routinely occurs in two-stage implant systems following placement of the abutment, thereby creating an IAI microgap in close approximation to the crestal bone level.^{1,3,4} In contrast, minimal bone loss has been observed when using a non-submerged single-stage implant system.¹⁻⁶

A recent study by Piatelli et al.⁵ examined the effect of microgap location on changes in crestal bone levels using a

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monkey model. Their results demonstrated a direct relationship between IAI microgap approximation to crestal bone and the amount of crestal bone resorption; i.e., as the microgap was moved coronally from crestal bone, the amount of bone loss decreased significantly. Other studies have demonstrated the possibility of leakage of proteins into and out of the IAI microgaps^{7,8} and bacterial colonization of microgap surfaces.^{2,9,10}

There are a number of studies suggesting detrimental effects of periodontopathic bacteria on peri-implant hard and soft tissue health.¹¹⁻¹⁴ However, there is limited knowledge available concerning the specific genus and species of bacteria that may colonize the internal surfaces of the IAI of two-stage dental implants *in situ*. Thus, the purpose of this preliminary study was to use DNA probe analysis to determine if periodontopathic bacteria inhabit the internal surfaces and/or the healing abutment screw-threads of the IAI of two-stage dental implants *in situ*.

MATERIALS AND METHODS

Study Population and Implants

The study population consisted of 32 private practice patients (17 females and 15 males), with an average age of 55.5 years. One patient was totally edentulous. Patients were recruited only if they exhibited excellent periodontal health; collectively, the study participants had <10% bleeding on probing of sites associated with the natural teeth. All study participants signed an informed consent acknowledging their voluntary participation in the study and assurance of complete anonymity. Patients were enrolled from January to November 2001.

A total of 54 two-stage hydroxyapatite plasma spray-coated implants from various manufacturers, 24 maxillary and 30 mandibular, were distributed among the patient population. Teeth were extracted for several reasons including endodontic problems, root fracture, and accidents. The average time lapse from implant insertion to the second-stage surgery and placement of the healing abutment was 147 days (5 months). Primary soft tissue closure using bioabsorbable sutures was attained over each implant at the time of insertion, and all implants exhibited clinical and radiographic evidence of complete osseointegration without complication. The average lapse of time from placement of the healing abutment to procurement of microbial samples was 25 days. Lastly, all healing abutments were seated using the torque-wrench provided by the implant manufacturers.

Sample Procurement and DNA Probe Analysis

Using sterile paper points provided in the DNA probe analysis kit,⁵ microbial samples were procured from the internal surface of the IAI of 43 implants and from the screw-threads of the healing abutment of 11 implants as follows: the implant site was isolated from saliva contamination by use of sterile 2 × 2 gauze pads; the heal-

ing abutment and surrounding soft tissues were carefully decontaminated by gentle scrubbing with sterile cotton balls soaked in a 10% solution of povidone-iodine; the healing abutment was then removed and the internal surface of the implant at the IAI was circumferentially wiped with the paper point, taking care to not encroach upon the internal screw-threads that connect the implant and abutment; and lastly, the healing abutments were wiped with a sterile paper point using a parallel orientation to the screw-threads.

The samples were submitted to the kit manufacturer's laboratory for bacterial identification using DNA probe analysis. DNA probes for the following eight putative periodontopathic bacteria were available: *Actinobacillus actinomycetemcomitans* (*A. actinomycetemcomitans*), *Tannerella forsythensis* (*T. forsythensis*), *Campylobacter rectus* (*C. rectus*), *Eikenella corrodens* (*E. corrodens*), *Fusobacterium nucleatum* (*F. nucleatum*), *Porphyromonas gingivalis* (*P. gingivalis*), *Prevotella intermedia* (*P. intermedia*), and *Treponema denticola* (*T. denticola*).

DNA probe analysis microbial pathogen level results were expressed as either negligible (less than 0.1% of total or fewer than 10³ cells); low (less than 0.1% to 0.9% of total or 10³ cells); medium (less than 0.1% to 9.9% of total or 10⁴ cells); or high (more than 10⁴ cells).

Data Analysis

Descriptive data were examined using frequency distributions, medians, and semi-interquartile ranges for the putative periodontopathic bacteria as well as implants with multiple microbial species. In addition, anterior (cuspid to cuspid) versus posterior and maxillary versus mandibular colonization of individual microbial species and IAI with multiple microbial species were examined using the Mann-Whitney U test.

RESULTS

All microbial samples obtained from screw-threads of the healing abutment (n = 11) were negligible (i.e., less than 0.1% of total or fewer than 10³ cells) for the presence of the bacteria. In contrast, 100% of the samples obtained from within implant internal surfaces of the IAI (n = 43) were positive for one or more of the target microbes. The aggregate percentage of positive results from the DNA probe analysis for each target microbe for samples taken from internal surfaces of the IAI is shown in Table 1. On average, approximately one-half of all implants were positive for each of the microbes, with a low of 41.9% for *A. actinomycetemcomitans* (18 of 43 samples) and a high of 60.5% for both *T. forsythensis* and *E. corrodens*.

Table 1 also shows the distribution of periodontopathic bacteria levels by the number of specimens (and percentage) obtained from internal surfaces of the IAI as determined by positive DNA probe analysis. A consistent

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Table 1.
Distribution of Bacteria by Number (%) of Positive Specimens
Obtained From Internal Surfaces of the IAI Interface (N = 43)

Bacteria	Negligible	Low	Medium	High	Aggregate % Positive Samples
<i>A. actinomycetemcomitans</i>	25 (58.1)	0	3 (7)	15 (34.9)	41.9
<i>T. forsythensis</i>	17 (39.5)	3 (7)	10 (23.3)	13 (30.2)	60.5
<i>C. rectus</i>	24 (55.8)	0	4 (9.3)	15 (34.9)	44.2
<i>E. corrodens</i>	17 (39.5)	0	13 (30.2)	13 (30.2)	60.5
<i>F. nucleatum</i>	22 (51.1)	0	11 (25.6)	10 (23.3)	48.8
<i>P. gingivalis</i>	23 (53.5)	0	11 (25.6)	9 (20.9)	46.5
<i>P. intermedia</i>	19 (44.2)	1 (2.3)	11 (25.6)	12 (27.9)	55.8
<i>T. denticola</i>	21 (48.8)	1 (2.3)	8 (18.6)	13 (30.2)	51.2

Table 2.
Distribution of IAI Internal Surfaces With
Multiple Microbial Species (N = 43)

Number Positive Bacterial Specimens	Number (%) of Implants
1	10 (23.3)
2	4 (9.3)
3	8 (18.6)
4	3 (7.0)
5	3 (7.0)
6	4 (9.3)
7	5 (11.6)
8	6 (14.0)

finding across most microbial species was the relatively high numbers of bacteria on positive implant surfaces. With the exception of *E. corrodens*, *F. nucleatum*, and *P. gingivalis*, all other positive implant surfaces had a greater percentage of high bacterial levels than moderate bacterial levels. *A. actinomycetemcomitans* levels ranged from 25 (58.1%) negligible specimens to 15 (34.9%) specimens exhibiting high levels, with *C. rectus* levels showing a similar trend. Approximately one-third of all implants (30.2) had high levels of *T. forsythensis*, *E. corrodens*, *P. intermedia*, and *T. denticola*.

Given the consistent association of *P. gingivalis*, *T. denticola*, and *T. forsythensis* with inflammatory periodontal disease,¹⁵ an analysis of the incidence of co-colonization of these microbes in the 43 samples yielded the following

results: none of the microbes were detected in nine specimens (21%); 14 specimens (32.5%) were positive for only one of the three microbes; six specimens (14%) were positive for two of the three microbes; and all three microbes were detected in 14 specimens (32.5%).

Table 2 displays the distribution of IAI surfaces positive for multiple microbial species. Only 23.3% of implant surfaces were positive for a single microbe, with approximately 50% having four or more of the cultured putative periodontopathic bacteria. Fourteen percent of the internal surfaces of the IAI were positive for all eight microbes.

Table 3 shows results for the medians and semi-interquartile (SI) ranges for levels of microbes and totals with multiple microbial species cultured by maxillary/mandibular gradient as well as anterior/posterior gradient. Although median (SI) levels for the periodontopathic bacteria varied somewhat between maxillary and mandibular sites, as well as anterior/posterior sites, the Mann-Whitney U test showed that these differences were not statistically significant ($P > 0.05$). The median number of IAI with multiple microbial species was similar for maxillary/mandibular sites (3.5 versus 3.0) and for anterior/posterior sites (3.0 versus 3.5). Neither of these comparisons was statistically significant ($P > 0.05$).

DISCUSSION

In general, longitudinal studies that evaluate dental implant success rates show that most failures occur within the first 2 years following insertion of the implant and after attachment of the implant abutment.¹⁶⁻¹⁹ Early bone loss and implant failure has been attributed to a variety of factors, such as surgical trauma during insertion,²⁰ occlusal overloading,²⁰⁻²² quality of supporting bone,^{20,23} smoking,^{20,24-26} peri-implantitis,^{20,27,28} and location of the microgap between the IAI.^{1-5,20}

In vitro studies have demonstrated leakage of fluids and penetration of the IAI microgap by bacteria.^{7,29} In addition, examination of failed implants by scanning electron microscopy have demonstrated the presence of bacteria on IAI surfaces.² Collectively, these studies included IAI microgaps ranging from $<10 \mu\text{m}$ to $>100 \mu\text{m}$.^{2,7,29} Thus, it would appear that microgaps at the IAI facilitate colonization of bacteria and offer shelter to the accumulated biofilm that, in turn, may lead to peri-implant inflammation. When seated according to manufacturers' recommendations, as done in the

Table 3.
Median (SI) Periodontopathic Bacteria Levels and Total With Multiple Microbial Species Cultured From Internal Surfaces of the IAI (N = 43)

Bacteria	Maxillary	Mandibular	Anterior	Posterior
<i>A. actinomycetem-comitans</i>	2.0 (1.5)	0 (1.5)	0 (1.5)	0 (1.5)
<i>T. forsythensis</i>	0.5 (1.5)	2.0 (1.5)	1.0 (1.5)	2.0 (1.5)
<i>C. rectus</i>	0 (1.5)	0 (1.5)	0 (1.5)	0 (1.5)
<i>E. corrodens</i>	2.0 (1.5)	2.0 (1.5)	0 (1.0)	2.0 (1.5)
<i>F. nucleatum</i>	2.0 (1.5)	0 (1.0)	2.0 (1.0)	0 (1.4)
<i>P. gingivalis</i>	0 (1.1)	2.0 (1.0)	2.0 (1.0)	0 (1.0)
<i>P. intermedia</i>	2.0 (1.5)	2.0 (1.5)	2.0 (1.5)	1.5 (1.5)
<i>T. denticola</i>	2.0 (1.5)	0 (1.5)	1.0 (1.0)	1.0 (1.5)
Total multiple microbial species	3.5 (2.5)	3.0 (2.75)	3.0 (3.0)	3.5 (2.4)

present study, microgaps between the implant and abutment are generally <10 μm . However, the diameter of a microbe averages <2.0 μm . Consequently, given access to the microgap, bacteria can easily penetrate even the tightest implant-abutment fitting. Further, off-axis occlusal loading of the implant may increase marginal opening at the IAI and thereby increase the potential for bacterial penetration and fluid percolation. A legitimate argument can be made that microgaps at or near bone level, e.g., two-stage implant systems, may present a greater risk for peri-implant bone loss due to bacterial colonization of the internal surfaces of the IAI.

In contrast to bacteria that colonize external surfaces of implants, those harbored within the IAI are protected from host defense mechanisms and may, therefore, persist for extended periods of time and create an environment conducive to recurring peri-implant infections.³⁰ The present study clearly demonstrated, via DNA probe analysis, the presence of periodontopathic microbes on smooth surfaces within the implant at the IAI. In addition, it should be noted that the target microbes were present, on average, at 25 days following the second-stage surgical exposure and placement of the healing abutment, in spite of the fact that all study subjects exhibited excellent oral hygiene and periodontal health. Thus, it would appear that an environment is established for microbial-induced peri-implant inflammation during the second stage surgery or shortly thereafter.

Composition of the oral bacterial flora prior to implant placement appears to dictate composition of the peri-implant bacterial population.³¹ In this regard, the bacterial flora associated with peri-implantitis resembles that of chronic or refractory periodontitis, e.g., significant levels of gram-negative anaerobic bacteria such as *Fusobacterium spp.*, *Treponema spp.*, *T. forsythensis*, *P. intermedia*, and *P. gingivalis*.³¹⁻³³ In the present study, moderate and high levels of eight putative periodontal pathogens were identified in all 43 implants (Table 2), with approximately 50% of the implants being positive for four or more pathogenic microbes. Given the excellent periodontal health of the study participants, one must consider that in addition to the normal tooth-associated microbial flora, secondary reservoirs such as dorsal tongue surfaces and peri-tonsillar areas may significantly contribute to colonization of the periodontopathic bacteria. It should be noted, however, that the mere presence of putative periodontal pathogens does not indicate a direct etiologic relationship that may lead to a destructive process but may simply indicate a potential pathogenic environment.^{35,36}

The presence of periodontopathic microbes on internal surfaces of the IAI is not surprising as several studies have demonstrated the translocation of bacteria from residual dentition to implants.^{37,38} In addition, Leonhardt, et al.³⁹ have reported the presence of putative periodontopathic microbes around dental implants in partially edentulous patients as early as one month after exposure to the oral cavity. The finding of negligible DNA probe results for all 11 samples taken from the screw-threads of the healing abutments is interesting and seems to indicate one of two possibilities: sufficient time had not elapsed after placement of the healing abutment for microbial penetration to the level of the screw-threads or the healing abutment screw and internal threads fit so tightly that microbial penetration was not possible.

Savitt et al.⁴⁰ compared DNA probe analysis to bacterial cultural methods and reported that probe analysis frequently identified periodontopathic microbes that were culture-negative. Indeed, DNA probe analysis on an individual basis produced better correlation between the presence of a pathogen and clinical evidence of disease. Baker et al.⁴¹ have noted that paper points, as used in this study, when used to sample periodontal pockets, attract microbes by capillary action and, thereby, preferentially select bacteria in crevicular fluid that are not strongly adherent to epithelium or root surface. The present study used paper points to physically rub and/or wipe the internal surfaces of the IAI and healing abutment screw-threads and did not rely on the presence of tissue or crevicular fluids. Although this technique does not assure that all adhering microbes are sampled, it is likely to achieve a more representative sampling than simply relying on capillary action.

This method of sampling could also account for the fact that all DNA probe analysis positive results were at either moderate or high levels for the target microbe.

In conclusion, moderate to high levels of eight different periodontopathic microbes inhabiting the internal surfaces of the IAI of 43 two-stage implants in partially edentulous patients were identified by DNA probe analysis. The microbes colonized these surfaces within 25 days following the second-stage surgery and placement of the healing abutment. In contrast, all samples obtained from screw-threads of 11 healing abutments were DNA probe negligible. These findings appear to support those of other investigations demonstrating the translocation of periodontopathic bacteria from residual dentition to implants.

REFERENCES

- Hermann JS, Cochran DL, Nummikoski PV, Buser D. Crestal bone changes around titanium implants: A radiographic evaluation of unloaded nonsubmerged and submerged implants in the canine mandible. *J Periodontol* 1997;68:1117-1130.
- Callan DP, O'Mahony A, Cobb CM. Loss of crestal bone around dental implants: A retrospective study. *Implant Dent* 1998;7:258-265.
- Hermann JS, Schoolfield JD, Buser D, Schenk RK, Cochran DL. Influence of the size of the microgap on crestal bone changes around titanium implants: A histometric evaluation of unloaded non-submerged implants in the canine mandible. *J Periodontol* 2001;72:1372-1383.
- King GN, Hermann JS, Schoolfield JD, Buser D, Cochran DL. Influence of the size of the microgap on crestal bone levels in non-submerged dental implants: A radiographic study in the canine mandible. *J Periodontol* 2002;73:1111-1117.
- Piatelli A, Vrespa G, Petrone G, Iezzi G, Annibali S, Scarano A. Role of the microgap between implant and abutment: A retrospective histologic evaluation of monkeys. *J Periodontol* 2003;74:346-352.
- Oh T-J, Yoon J, Misch CE, Wang H-L. The causes of early implant bone loss: Myth or science. *J Periodontol* 2002;73:322-333.
- Gross M, Abramovich I, Weiss EI. Microleakage at the abutment-implant interface of osseointegrated implants: A comparative study. *Int J Oral Maxillofac Implants* 1999;14:94-100.
- Guindy JS, Besimo CE, Besimo R, Schiel H, Meyer J. Bacterial leakage into and from prefabricated screw-retained implant-borne crowns in vitro. *J Oral Rehab* 1998;25:403-408.
- Quirynen M, van Steenberghe D. Bacterial colonization of the internal part of two-stage implants. An in vivo study. *Clin Oral Implants Res* 1993;4:158-161.
- Persson LG, Lekholm U, Leonhardt A, Dahlen G, Lindhe J. Bacterial colonization on internal surfaces of Brånemark system implant components. *Clin Oral Implants Res* 1996;7:90-95.
- Heydenrijk K, Meijer HJ, van der Reijden WA, Raghoobar GM, Vissink A, Stegenga B. Microbiota around root-form osseous implants: A review of the literature. *Int J Oral Maxillofac Implants* 2002;17:829-838.
- Hultin M, Gustafsson A, Hallstrom H, Johansson LA, Ekfeldt A, Klinge B. Microbiological findings and host response in patients with peri-implantitis. *Clin Oral Implants Res* 2002;13:349-358.
- Rutar A, Lang NP, Buser D, Burgin W, Mombelli A. Retrospective assessment of clinical and microbiological factors affecting periimplant tissue conditions. *Clin Oral Implants Res* 2001;12:189-195.
- van Winkelhoff AJ, Goene RJ, Benschop C, Folmer T. Early colonization of dental implants by putative periodontal pathogens in partially edentulous patients. *Clin Oral Implants Res* 2000;11:511-520.
- Listgarten MA, Loomer PM. Microbial identification in the management of periodontal diseases. A systematic review. *Ann Periodontol* 2003;8:182-192.
- Pylant T, Triplett RG, Key MC, Brunsvold MA. A retrospective evaluation of endosseous titanium implants in the partially edentulous patient. *Int J Oral Maxillofac Implants* 1992;7:195-202.
- Naert I, Quirynen M, van Steenberghe D, Darius P. A six-year prosthodontic study of 509 consecutively inserted implants for the treatment of partial edentulism. *J Prosthet Dent* 1992;67:236-245.
- Wheeler SL. Eight-year clinical retrospective study of titanium plasma-sprayed and hydroxyapatite-coated cylinder implants. *Int J Oral Maxillofac Implants* 1996;11:340-350.
- Lee JJ, Roubfar L, Beirne OR. Survival of hydroxyapatite-coated implants: A meta-analytic review. *J Oral Maxillofac Surg* 2000;58:1372-1379.
- Esposito M, Hirsch J-M, Lekholm U, Thomsen P. Biological factors contributing to failures of osseointegrated oral implants. (II). Etiopathogenesis. *Eur J Oral Sci* 1998;106:721-764.
- Quirynen M, Naert I, van Steenberghe D. Fixture design and overload influence marginal bone loss and fixture success in the Brånemark system. *Clin Oral Implants Res* 1992;3:104-111.
- Isidor F. Histological evaluation of peri-implant bone at implants subjected to occlusal overload or plaque accumulation. *Clin Oral Implants Res* 1997;8:1-9.
- Truhlar RS, Farish SE, Scheitler LE, Morris HF, Ochi S. Bone quality and implant design-related outcomes through stage II surgical uncovering of spectra-system root form implants. *J Oral Maxillofac Surg* 1997;55 (Suppl. 5):46-54.
- Wallace RH. The relationship between cigarette smoking and dental implant failure. *Eur J Prosthodont Rest Dent* 2000;8:103-106.
- van Steenberghe D, Jacobs R, Desnyder M, Maffei G, Quirynen M. The relative impact of local and endogenous patient-related factors on implant failure up to the abutment stage. *Clin Oral Implants Res* 2002;13:617-622.
- Schwartz-Arad D, Samet N, Samet N, Mamlider A. Smoking and complications of endosseous dental implants. *J Periodontol* 2002;73:153-157.
- 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.
- Marinello CP, Berglundh T, Ericsson I, Klinge B, Glantz PO, Lindhe J. Resolution of ligature-induced peri-implantitis lesions in the dog. *J Clin Periodontol* 1995;22:475-479.
- Jansen VK, Conrads G, Richter E-J. Microbial leakage and marginal fit of the implant-abutment interface. *Int J Oral Maxillofac Implants* 1997;12:527-540.
- Listgarten MA. Microorganisms and dental implants [guest editorial]. *J Periodontol* 1999;70:220-222.

31. Heydenrijk K, Meijer HJ, van der Reijden WA, Raghoobar GM, Vissink A, Stegenga B. Microbiota around root-form endosseous implants: A review of the literature. *Int J Oral Maxillofac Implants* 2002;17:829-838.
32. Listgarten MA, Lai CH. Comparative microbiological characteristics of failing implants and periodontally diseased teeth. *J Periodontol* 1999;70:431-437.
33. Mombelli A, van Oosten MAC, Schürch E, Lang NP. The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiol Immunol* 1987;2:145-151.
34. Mombelli A, Lang NP. Microbial aspect of implant dentistry. *Periodontology* 2000 1994;4:74-80.
35. Wennström JL, Dahlén G, Svensson J, Nyman S. *Actinobacillus actinomycetemcomitans*, *Bacteroides gingivalis*, *Bacteroides intermedius*: Predictors of attachment loss? *Oral Microbiol Immunol* 1987;2:158-162.
36. Dzink JL, Tanner AC, Haffajee AD, Socransky SS. Gram negative species associated with active destructive periodontal lesions. *J Clin Periodontol* 1985;12:648-659.
37. Kohavi D, Greenberg R, Raviv E, Sela MN. Subgingival and supragingival microbial flora around healthy osseointegrated implants in partially edentulous patients. *Int J Oral Maxillofac Implants* 1994;9:673-678.
38. Mombelli A, Marker M, Gaberthüel T, Grunder U, Lang NP. The microbiota of osseointegrated implants in patients with a history of periodontal disease. *J Clin Periodontol* 1995;22:124-130.
39. Leonhardt A, Adolfsson B, Lekholm U, Wikström M, Dahlén G. A longitudinal microbiological study on osseointegrated titanium implants in partially edentulous patients. *Clin Oral Implants Res* 1993;4:113-120.
40. Savitt ED, Strzempko MN, Vaccaro KK, Peros WJ, French CK. Comparison of cultural methods and DNA probe analyses for the detection of *Actinobacillus actinomycetemcomitans*, *Bacteroides gingivalis* and *Bacteroides intermedius* in subgingival plaque samples. *J Periodontol* 1988;59:431-438.
41. Baker PJ, Butler R, Wikesjö UME. Bacterial sampling by absorbent paper points. An in vitro study. *J Periodontol* 1991;62:142-146.

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