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The evaluation of bacterial flora in progress of peri-implant disease

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ABSTRACT

Background: Cumulative interceptive supportive therapy (CIST) is currently used as a guideline for treating peri-implant diseases. The objectives of this study were to determine the detection rate and measure the number of periodontopathic bacteria in lesions of different CIST levels and thereby characterize peri-implant disease from a bacteriological viewpoint.
Methods: This study included 105 patients who had both residual natural teeth and implants with peri-implant disease. A total of 105 implants were divided into levels A, B, C and D according to the CIST classification. Bacterial samples were collected from peri-implant pockets and four periodontopathic bacteria were measured by PCR and PCR-Invader assay.
Results: The number of periodontopathic bacteria increased in line with CIST level, and the detection rate was also associated with CIST level. However, no difference was found in the bacterial detection rate of *P. gingivalis* and *T. denticola* between CIST-B and CIST-C. There was a higher detection rate of all periodontopathic bacteria for CIST-D.
Conclusions: The number of periodontopathic bacteria and detection rate increased as peri-implant disease advanced. However, there were no major differences in the detection rate between CIST-B and CIST-C. On the other hand, a higher detection rate of periodontopathic bacteria was seen for CIST-D.

Keywords: Peri-implantitis, implant, bacteria, flora, treatment failure.

Abbreviations and acronyms: BOP = bleeding on probing; CIST = cumulative interceptive supportive therapy; PCR = polymerase chain reaction; PPD = probing pocket depth.

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INTRODUCTION

It is known that implants are affected by pathogenic bacteria from teeth with existing periodontal disease in the same oral cavity. The detection rate of representative periodontopathic bacteria, including *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythensis* and *Aggregatibacter actinomycetemcomitans*, in peri-implant pockets has been shown to be very similar to that in periodontal pockets of residual teeth.[1,2] Previous studies comparing the gene expression pattern of these bacteria showed that bacteria detected from peri-implant and periodontal pockets frequently exhibit an identical pattern of gene expression, suggesting transmission of bacteria from periodontal pockets around residual natural teeth into peri-implant pockets.[3,4] Thus, the condition of implants appears to be strongly affected by the periodontal status of the residual natural teeth. Studies using Brånemark implants[5] and ITI implants[6] have shown higher incidence of peri-implantitis and bone resorption in patients with periodontal disease than in those without. Meanwhile, a number of studies have demonstrated a consistent success rate of implants and stable maintenance of implants even in those patients who had lost their teeth due to periodontal disease.[7–10]

Mombelli and Lang[11] proposed cumulative interceptive supportive therapy (CIST) as a guideline for treating implants affected by peri-implant disease (Fig 1). In this therapy, treatment is classified into classes A to D according to the extent of progression of peri-implant disease. The classification is based on the depth of peri-implant pockets with or without bleeding on probing (BOP) and on the extent of bone resorption, and treatment is specified for each class. The details are as follows: (1) lesions with deposition of plaque and dental calculus on the implant, positive for BOP, no pus discharge and probing pocket depth (PPD) of less than 4 mm are classified as level A and treated with mechanical debridement. More specifically, dental

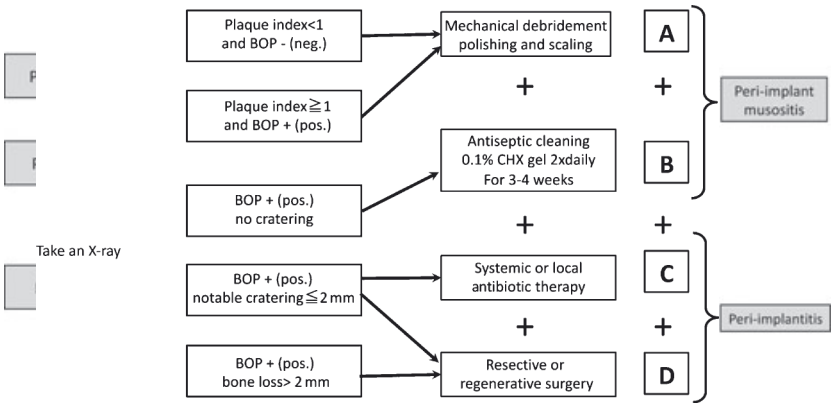

Fig 1. Classification of cumulative interceptive therapy (CIST). CIST-A and CIST-B show peri-implant mucositis; CIST-C and CIST-D show peri-implantitis.

calculus is removed with a plastic curette or other tools so as not to damage the implant surface; (2) lesions positive for BOP and with PPD of 4 to less than 5 mm are classified as level B and treated with mechanical debridement and a disinfectant. More specifically, 0.1–0.2% chlorhexidine gluconate solution will be prescribed as a mouth cleanser or 0.2% gel of the agent will be applied to the lesion; (3) lesions positive for BOP and with PPD of 5 mm or more and bone resorption of 2 mm or less on the X-ray are classified as level C and treated with systemic or local antibiotics in addition to the procedures used for level B lesions; and (4) lesions positive for BOP and with PPD of 5 mm or more and bone resorption of 2 mm or more on the X-ray are classified as level D and treated with the same regimen used for level C lesions until inflammation is resolved, followed by either resection or regeneration therapy. As described above, treatment regimens are specified for each CIST level. However, the composition of periodontopathic bacteria in peri-implant pockets for each CIST level has not yet been elucidated.

The objectives of the present study were to evaluate the detection rate and the number of periodontopathic bacteria in lesions of each CIST level and thereby characterize peri-implant disease from a bacteriological viewpoint.

MATERIALS AND METHODS

This study was undertaken at the Department of Periodontics and Division of Oral and Maxillofacial Implantology, Tsurumi University Dental Hospital, Japan. The Ethics Committee approved the trial, and all patients gave written informed consent before the study commenced.

A total of 105 subjects (42 males and 63 females; mean age 53.7 ± 15.9 years) who had both residual natural teeth and implants with peri-implantitis were selected from patients referred to the Tsurumi University Dental Hospital. Subjects had no systemic diseases and had received no antibiotics within the past six months. In addition, no subjects had received active periodontal treatment with the exception of scaling within the past four years. Moreover, the periodontal condition of their residual teeth was stable.

As a clinical parameter for peri-implant disease, the depth of the peri-implant pocket was measured with a Williams probe (Hu-Friedy, Chicago, IL, USA). Other parameters evaluated were plaque index (Sillnaee and Löe),[12] BOP, pus discharge and tooth mobility (Miller).[13] The status of the bone defect was assessed by dental X-ray imaging. Traumatic occlusions were not observed in examined implants. In reference to the results of the above examinations, 105 implants in 105 subjects were divided into levels A, B, C and D according to the CIST classification. The 105 implants (Brånemark: 52; ITI: 30; ASTRA: 7 and unknown: 16) were placed more than five years after implantation. The distribution of implants was as follows: 21 implants in CIST-A; 38 implants in CIST-B; 17 implants in CIST-C and 29 implants in CIST-D. All residual teeth were examined by PPD and BOP. To collect bacteria from peri-implant pockets and residual teeth, a sterile paper point #40 (PIERCE, Tokyo, Japan) was inserted to the bottom of a pocket, left in place for 30 seconds, transferred into a serum tube containing 1 ml of pure water and stored at –20 °C. Each sample was sonicated for 30 seconds with a BRANSON2510 (Yamato, Tokyo, Japan) and used for the detection of the following four species of periodontopathic bacteria: *P. gingivalis, T. denticola, T. forsythensis* and *A.* (*Actinobacillus*) *actinomycetemcomitans.*

Determination of bacterial detection rate

The four species of periodontopathic bacteria, *T. forsythensis*, *T. denticola*, *P. gingivalis* and *A. actinomycetemcomitans*, were identified in each subgingival sample by conventional polymerase chain reaction (PCR) as described below.[14] The samples were

kept at 4 °C in a transport medium, mixed well on a Vortex mixer (Vortex-Genie 2, Scientific Industries Inc, NY, USA) and treated by ultrasonication (Branson 2510, Yamato, Japan) at 100 W for 10 minutes. The microbial suspension samples were then boiled at 99 °C for 10 minutes by heat-block (Dry Thermo Unit DTU-1B, Taitec Corporation, Japan) and kept at 4 °C. PCR was performed using 4.8 μl of the sample added to 5.2 μl of reaction mixture that contained 5 μl of the HotStar Taq Master Mix Kit (Qiagen, Japan) and sense and antisense primer. Species-specific PCR primers for the four bacteria were designed by Ashimoto *et al.*[12] All primers were synthesized by a commercial company (Bex Co. Ltd, Japan). PCR amplification was performed in a DNA thermal cycler (GeneAmp PCR System 9700, Applied Biosystems, Japan). The PCR temperature profile for *P. gingivalis*, *T. forthysia* and *T. denticola* included initial denaturation at 95 °C for 2 minutes, followed by 36 cycles of denaturation at 95 °C for 30 seconds, primer annealing at 60 °C for 1 minute, extension at 72 °C for 1 minute and a final extension step of 72 °C for 2 minutes. The temperature profile for *A. actinomycetemcomitans* included an initial step of 95 °C for 2 minutes followed by 36 cycles of denaturation at 94 °C for 30 seconds, primer annealing at 55 °C for 1 minute, extension at 72 °C for 2 minutes, and a final extension step of 72 °C for 10 minutes. PCR products were analysed by 1.5% agarose gel electrophoresis at 100 V in Tris-acetate EDTA buffer. The gel was stained with 0.1 μg/ml ethidium bromide. The washed gel was visualized by ultraviolet fluorescence at 312 nm using a Densitograph AE-6920 (Atto, Tokyo, Japan), and bands specific to the four bacterial species were detected using a Lane & Spot Analyser (Atto).

Quantitative analysis by PCR-invader assay

Ten implants were randomly selected from each group and subjected to quantitative and qualitative analyses by PCR-Invader® assay (BML Inc., Tokyo, Japan).[15] Bacterial DNA was purified using MagNA Pure LC (Roche, Basel, Switzerland) and added to a 15-μl reaction mixture containing 50 μM d-NTP, 700 nM primer probe, 70 nM Invader oligo, and 2.5 U AmpliTaq Stoffel fragment (Applied Biosystems, Foster City, CA, USA), and the Cleavase XI Invader core re-agent kit (Genomic DNA, Third Wave Technologies Inc., Madison, WI, USA) consisting of Cleavase XI FRET mix and Cleavase XI enzyme/$MgCl_2$ solution. The reaction mixture was preheated at 95 °C for 2 minutes, followed by 35 cycles of two-step PCR (95 °C for 1 minute, 63 °C for 1 minute) using Light-Cycler 480 (Roche, Basel, Switzerland). The fluorescence values of carboxyfluorescein were measured at the end of the incubation/extension step at 63 °C for each cycle. The standard curve was constructed by dilutions of plasmids containing the amplified regions. The above analyses were outsourced to a clinical laboratory test company (BML Inc., Tokyo, Japan).

Statistical analysis

Statistical analysis was performed using SPSS Version 14J (SPSS Japan Inc., Tokyo, Japan). Clinical parameters and the detection rate of periodontopathic bacteria were analysed using the one-way ANOVA. The number of periodontopathic bacteria was analysed using the Mann-Whitney U-test. The level of significance was set at 0.05.

RESULTS

CIST classification and distribution of the implant type indicated the following: CIST-A (Brånemark: 10, ITI: 8, ASTRA: 1, unknown: 2); CIST-B (Brånemark: 19, ITI: 10, ASTRA: 2, unknown: 7); CIST-C (Brånemark: 10, ITI: 5, ASTRA: 1, unknown: 1); and CIST-D (Brånemark: 13, ITI: 7, ASTRA: 3, unknown: 6). The mean PPD of the residual natural teeth was 1.9 ± 0.6 mm in patients with CIST-A implants, 2.5 ± 0.9 mm for CIST-B, 2.6 ± 1.0 mm for CIST-C and 2.6 ± 0.7 mm for CIST-D. Although the CIST-A group had relatively low values, no significant difference was observed for this parameter among the groups. On the other hand, the mean depth of peri-implant pockets was 3.1 ± 0.4 mm for CIST-A, 4.4 ± 0.4 mm for CIST-B, 6.6 ± 1.2 mm for CIST-C and 7.3 ± 1.8 mm for CIST-D, showing significant differences among groups (Fig 2). The BOP rate was expressed as a percentage of BOP-positive sites in all examined sites. The BOP rates for residual natural teeth in patients with CIST-A, CIST-B, CIST-C and CIST-D implants were 3.5%, 3.8%, 4.2% and 7.1%, respectively, while those for CIST-A, CIST-B, CIST-C

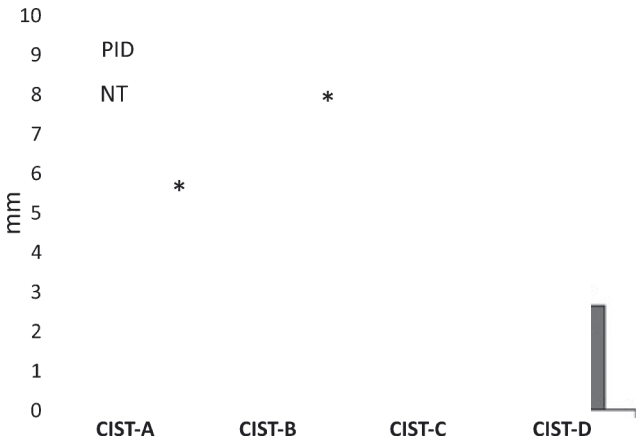

Fig 2. Mean PPD of residual natural teeth (NT) was less than 3 mm in all CIST levels. However, the implant circumference pocket (PID) was significantly deepened with progress of CIST level (*$p < 0.05$) (one-way ANOVA).

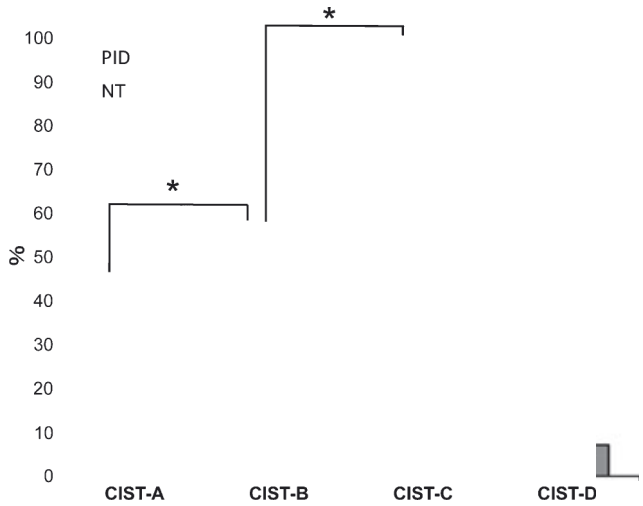

Fig 3. The BOP rate of residual natural teeth (NT) was under 10% in all CIST classes. The BOP rate of peri-implant disease (PID) significantly increased with the CIST level (*p < 0.05) (one-way ANOVA).

and CIST-D implants were 14.3%, 47.4%, 100% and 100%, respectively. The BOP rate was thus significantly higher for implants than for natural teeth (Fig 3). The plaque index of all groups of implants and residual teeth was from 1.2 to 1.6, with no significant differences between them. The mobility of implants and residual teeth were all zero.

Changes in periodontopathic bacteria in peri-implant pockets as evaluated by PCR

The detection rate of bacteria in each CIST group as determined by PCR is shown in Fig 4. The detection rates of *P. gingivalis* in the CIST-A, CIST-B, CIST-C and CIST-D groups were 4.8%, 30.9%, 25.0% and 86.2%, and those of *T. denticola* were 9.5%, 36.3%, 33.3% and 75.9%, respectively. The CIST-D group showed higher detection rates of *P. gingivalis* and *T. denticola* compared to the other groups, while no significant difference was observed in the detection rates of these bacteria between CIST-B and CIST-C. On the other hand, the detection rate of *T. forsythensis* increased with the severity of peri-implant disease: 9.5%, 27.2%, 40.0% and 75.9% in the CIST-A, CIST-B, CIST-C and CIST-D groups, respectively. A higher detection rate of periodontopathic bacteria was found for CIST-D, which was characterized by deeper bone defects. *A. actinomycetemcomitans* was detected only in the CIST-D group, at a rate of 13.8%.

Quantitative analysis by PCR-Invader assay

The numbers of periodontopathic bacteria in each CIST group as measured by PCR-Invader assay are shown in Fig 5. The total numbers of bacteria in CIST-A, B, C and D groups were 8.6×10^5, 7.1×10^5, 9.2×10^6 and

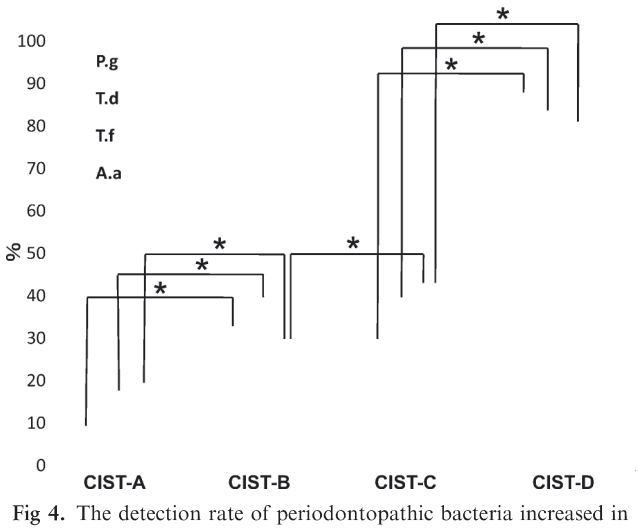

Fig 4. The detection rate of periodontopathic bacteria increased in line with CIST level. However, no significant difference in detection rate of *P. gingivalis* and *T. denticola* between CIST-B and CIST-C ($p < 0.05$) was observed. CIST-D, characterized by deeper bone defects, indicated a higher detection rate of periodontopathic bacteria. *A. actinomycetemcomitans* was detected only in CIST-D (one-way ANOVA).

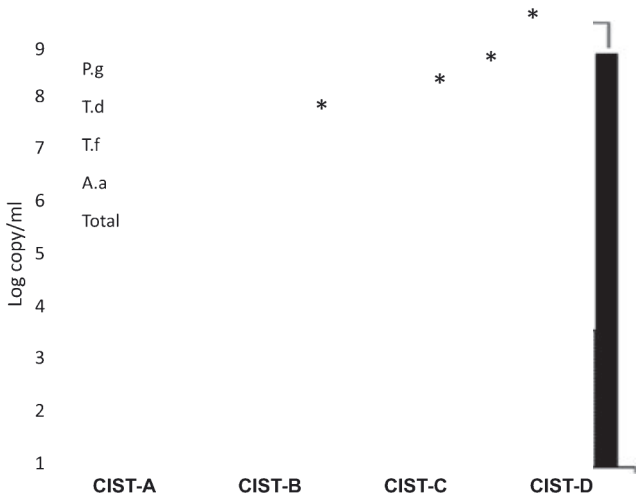

Fig 5. The number of periodontopathic bacteria, except *T. forsythensis*, significantly increased in line with CIST level ($p < 0.05$) between CIST-C and CIST-D (Mann-Whitney U-test).

8.0×10^7 copy/ml, respectively. The total number of bacteria increased as the severity of peri-implantitis increased from CIST-A to CIST-D. The numbers of *P. gingivalis* in the CIST-A, CIST-B, CIST-C and CIST-D groups were 0, 9.3×10^3, 6×10^5 and 3×10^6 copy/ml. Those of *T. denticola* were 0, 3.4×10^2, 1.9×10^4 and 7.5×10^4 copy/ml; and those of *T. forsythensis* were 0, 1×10^4, 2.7×10^5 and 8.2×10^5 copy/ml, respectively. *A. actinomycetemcomitans* was detected only in the CIST-D group, at a count of 4.1×10^2 copy/ml. The numbers of periodontopathic bacteria significantly increased in line with CIST level, which indicated the progression of peri-implant disease.

DISCUSSION

The present study aimed to evaluate the detection rate and measure the quantity of periodontopathic bacteria in lesions of each CIST level as a cross-sectional study and thereby characterize peri-implant disease from a bacteriological viewpoint. To date, the composition of periodontopathic bacteria in the peri-implant pockets of each CIST level has not yet been elucidated.

Many studies have reported that peri-implant microflora is strongly affected by the microflora of residual teeth with periodontitis.[16–18] Our view on such findings is that it is possible to incorrectly evaluate the bacterial flora of peri-implant pockets in the progression of peri-implant disease. Moreover, it is vital to understand the characteristics of bacterial flora of peri-implant disease without the effects of bacterial flora of residual teeth with periodontitis. In this study, the PPDs of residual teeth were, although relatively lower in CIST-A than in other groups, also not significantly different among groups (average pocket depth of CIST-A to CIST-E was from 1.9 to 2.6 mm). Similarly, the BOP rates of residual natural teeth were also not significantly different among groups. In particular, the conditions of periodontal disease in residual teeth were similar regardless of CIST level, and the condition of the periodontium was stable. This suggests that the bacterial evaluation of peri-implant diseases was affected less by the conditions of periodontal disease in residual teeth. Therefore, it may be valuable to evaluate the progression of peri-implant disease from a more bacteriological viewpoint.

Mombelli and associates[19] isolated an increased proportion of Gram-negative anaerobic rods in edentulous and partially edentulous patients, especially *P. intermedia, Fusobacteria* and *T. denticola*. Listgarten and Lai[20] isolated *T. forsythensis*, *T. denticola*, *Fusobacterium* and *P. gingivalis* around many of the failing implants in partially edentulous patients. Similarly, in our study, *T. forsythensis*, *T. denticola* and *P. gingivalis* were detected in each CIST level, and it was confirmed that periodontal pathogens were strongly associated with progression of peri-implant disease. Listgarten and Lai[20] also reported the detection rates of *P. gingivalis* and *T. forsythensis*, as 27% and 59%, respectively. These occurrence rates were close to our data for CIST-C (25% and 40%, respectively). Our research compared with past results showed similar results. We examined the bacterial detection rate according to classification of CIST. The detection rate of bacteria determined by PCR increased as peri-implant disease advanced, but exhibited no significant difference in *P. gingivalis* and *T. denticola* between CIST-B and CIST-C. This study was limited and performed with a small sample population. However, PCR-Invader assay revealed an increase in the number of periodontopathic bacteria in line with significant increases according to CIST level. Furthermore, a significant difference was found in bacterial number between CIST-B and CIST-C.

From these results, we surmised that the increase of periodontopathic bacteria played an important role during the transition from peri-implant mucositis (CIST-B) to peri-implantitis (CIST-C). Additionally, as CIST-C showed angular alveolar bone resorption, it was thought that bone resorption was induced by an increase of periodontopathic bacteria in peri-implant pockets. CIST-D, characterized by deeper bone defects, indicated a higher detection rate of periodontopathic bacteria.

When peri-implantitis is established, the location of periodontal pathogen growth is enlarged by a deepening pocket and bone resorption, and the detection rate and number of periodontopathic bacteria are increased. *A. actinomycetemcomitans* was detected from CIST-D lesions with severe peri-implant destruction. Quirynen *et al.*[21] have shown that *A. actinomycetemcomitans* is frequently detected in patients with apparent peri-implant bone resorption, which is consistent with the present study. Of the red complex[22] detected by PCR, *T. forsythensis* and *T. denticola* were not detected by PCR-Invader assay in the CIST-A group, possibly because of the difficulty sampling from shallow pockets such as the CIST-A pocket.

CONCLUSIONS

Both the number and detection rate of periodontopathic bacteria were associated with CIST level. However, there were no major differences in the detection rate between CIST-B and CIST-C. Thus, it is thought that the quantitative increase of periodontal pathogens was strongly associated with the transition from peri-implant mucositis to peri-implantitis. On the other hand, CIST-D showed a higher detection rate of periodontopathic bacteria. The present findings demonstrate that strong relations exist between periodontal pathogens and peri-implantitis.

REFERENCES

1. Lekholm U, Ericsson I, Adell R, Slots J. The condition of the soft tissues at tooth and fixture abutments supporting fixed bridges. A microbiological and histological study. J Clin Periodontol 1986;13:558–562.
2. Luterbacher S, Mayfield L, Bragger U, Lang NP. Diagnostic characteristic of clinical and microbiological tests for monitoring periodontal and peri-implant mucosal tissue conditions during supportive periodontal therapy (SPT). Clin Oral Implants Res 2000;11:521–529.
3. Sumida S, Ishihara K, Kishi M. Transmission of periodontal disease associated bacteria from teeth to osseointegrated implant regions. Int J Oral Maxillofac Implants 2002;17:696–702.

4. Ogawa H, Ohsima T, Kawasaki F. Periopathic bacterial transmission from periodontal pockets of natural teeth to peri-implant sulci (in Japanese). J Japanese Oral Implantology 2005;18:556–562.
5. Hardt CR, Gröndahl K, Lekholm U, Wennström 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.
6. Karoussis IK, Salvi GE, Heitz-Mayfield LJ, Brägger U, Hämmerle 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.
7. Nevins M, Langer B. The successful use of osseointegrated implants for the treatment of the recalcitrant periodontal patient. J Periodontol 1995;66:150–157.
8. Mengel R, Stelzel M, Hasse C, Flores-de-Jacoby L. Osseointegrated implants in patients treated for generalized severe adult periodontitis. An interim report. J Periodontol 1996;67:782–787.
9. Quirynen M, Peeters W, Naert I, Coucke W, van Steenberghe D. Peri-implant health around screw-shaped c.p. titanium machined implants in partially edentulous patients with or without ongoing periodontitis. Clin Oral Implants Res 2001;12:589–594.
10. Baelum V, Ellegaard B. Implant survival in periodontally compromised patients. J Periodontol 2004;75:1404–1412.
11. Mombelli A, Lang NP. The diagnosis and treatment of peri-implantitis. Periodontol 2000 1998;17:63–76.
12. Sillness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121–135.
13. Miller SC. Describes the most common method for assessing mobility. Textbook of Periodontia. 3rd edn. Philadelphia: The Blakeston Co., 1950:125.
14. 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.
15. Tadokoro K, Yamaguchi T, Shimizu H, *et al.* Rapid quantification of periodontitis-related bacteria using a novel modification of Invader PLUS technology. Microbiol Res 2010;165:43–49.
16. 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.
17. Quirynen M, Listgarten MA. Distribution of bacterial morphotypes around natural teeth and titanium implants ad modum Brånemark. Clin Oral Implants Res 1990;1:8–12.
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. Mombelli A, van Oosten M, Schurch E, Lang NP. The microbiota associated with successful or failing osseointegrated titanium implants. Oral Microbiol Immunol 1987;2:145–151.
20. Listgarten MA, Lai CH. Comparative microbiological characteristics of failing implants and periodontally diseased teeth. J Periodontol 1999;70:431–437.
21. 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.
22. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RI Jr. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25:766–775.

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