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<td>Author(s)</td>
<td>Aoki, M; Takanashi, K; Matsukubo, T; Yajima, Y; Okuda, K; Sato, T; Ishihara, K</td>
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Transmission of Periodontopathic Bacteria from Natural Teeth to Implants

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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 periimplant 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, P. intermedia, P. gingivalis, T. denticola and F. nucleatum in the gingival crevices of adjacent teeth and that of the peri-implant sulcus, but no association for T. forsythia.

Conclusions: The present findings suggest that colonization by A.
actinomycetemcomitans, P. intermedia, P. gingivalis, T. 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.
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 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 since 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 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. Branemark, ITI and Ankylos implants 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 second-stage surgery. In a previous study\(^\text{18}\), we found that \textit{P. gingivalis} displayed a 63.7\% detection rate in the implant sulcus one 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\(^\text{19-22}\). Based on these reports, samples from the implant sulci were obtained 2 weeks after second-stage surgery. The collected plaque samples were suspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and microorganisms harvested by centrifugation at 16,000 x g at 4\(^\circ\)C for 10 min. The pellets were then stored at -20\(^\circ\)C for subsequent detection of periodontal bacteria.
Detection of periodontopathic bacteria by the polymerase chain reaction (PCR) using specific primers designed from 16s RNA 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 min. Genomic DNA was isolated as described previously. Detection of Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia and Fusobacterium nucleatum was performed by PCR as described previously in a thermal cycler (Gene Amp PCR system 9700, PE Biosystems, Foster City, CA) 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).

Statistical analysis

In the stepwise logistic analysis, the Windows SAS9.1 software was used to evaluate the relationship between colonization by periodontopathic bacteria in the gingival crevices 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.

Results

Detection rates of for *actinomycescomitans*, *P. intermedia*, *P. gingivalis*, *T. denticola*, *T. 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 (Fig. 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 *P. gingivalis* and *T. denticola* for the adjacent teeth were higher than those at the occluding or contralateral teeth. The detection rate of *P. 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. actinomycescomitans* at the adjacent teeth was somewhat higher than that at the occluding or contralateral teeth. The detection rates for *T. 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*, *P. intermedia*, *P. gingivalis*, *T. denticola* and *F. nucleatum* from implant sulcus was correlated with detection from the gingival crevices of adjacent teeth. Detection of *T. 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*, *P. intermedia*, *P. gingivalis*, *T. 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 the detection profiles of *P. gingivalis*, *A. actinomycetemcomitans*, *T. denticola*, *T. forsythia* and *P. intermedia* in peri-implant
sulci were similar to those in the gingival crevice of natural teeth\textsuperscript{23}. In addition, clonal-type \textit{P. gingivalis} and \textit{P. intermedia} were isolated from both peri-implant sulci and the gingival crevices of natural teeth\textsuperscript{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. \textit{P. gingivalis} was reported to adhere to the tooth surface by attachment of fimbriae to salivary proline-rich protein and statherin\textsuperscript{28}. \textit{T. denticola} and \textit{F. nucleatum} colonize dental plaque biofilm by coaggregating with several species of plaque microorganisms\textsuperscript{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 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.

*P. gingivalis* and *A. actinomycetemcomitans* showed a high odds ratio among the periodontopathic bacteria investigated. These two microorganisms have fimbriae as structures for adherence. Fimbriae-deficient mutants of both species were reported to show reduced virulence. 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 *P. gingivalis*. *F. nucleatum* was detected not only in adults, but also in children. This fastidious microorganism also showed the highest prevalence in the present study. This may be why the correlation between the other species tested with implants and adjacent teeth was relatively low. No correlation was observed between detection of *T. forsythia* in peri-implant sulci and in the gingival crevices of natural 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 due to peri-implantitis.

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Table 1. List of species-specific and ubiquitous primers for PCR to detect 6 periodontopathic bacteria targeted.

<table>
<thead>
<tr>
<th>Primer pairs (5'-3')</th>
<th>Amplicon length in bp</th>
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<tr>
<td><strong>Aggregatibacter actinomycetemcomitans</strong></td>
<td></td>
</tr>
<tr>
<td>AAA CCC ATC TCT GAG TTC TTC TTC ATG CCAACT TGA CGT TAAAT</td>
<td>557</td>
</tr>
<tr>
<td><strong>Prevotella intermedia</strong></td>
<td></td>
</tr>
<tr>
<td>TTT GTT GGG GAG TAAAGC GGG TCAACA TCT CTG TGG GCT GCG T</td>
<td>575</td>
</tr>
<tr>
<td><strong>Porphyromonas gingivalis</strong></td>
<td></td>
</tr>
<tr>
<td>AGG CGA CTT GCC ATA CTG CG ACT GTT AGCAAC TAC CGA TGT</td>
<td>404</td>
</tr>
<tr>
<td><strong>Treponema denticola</strong></td>
<td></td>
</tr>
<tr>
<td>TAA TAC CGAAGC TCA TTT ACA T TCAAAG TCT CTG TGG GCT GCG A</td>
<td>316</td>
</tr>
<tr>
<td><strong>Tannerella forsythia</strong></td>
<td></td>
</tr>
<tr>
<td>GCG TAT GTAACC TGC CCG CA TGC TTC AGT GTG AGT TAT ACC T</td>
<td>641</td>
</tr>
<tr>
<td><strong>Fusobacterium nucleatum</strong></td>
<td></td>
</tr>
<tr>
<td>CTG AAC ATT GGAAC TAT ATA GTA GAACAA ACAAG GTC CTT CAT CGG CTC TTA CTA CCT AGG C</td>
<td>142</td>
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Table 2. Detection rate of periodontopathic bacteria from both implants and adjacent, occluding or contralateral teeth.

<table>
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<th>Teeth</th>
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<tr>
<td></td>
<td>Adjacent</td>
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<tr>
<td><em>A. actinomycetemcomitans</em></td>
<td>23.8</td>
</tr>
<tr>
<td><em>P. intermedia</em></td>
<td>38.1</td>
</tr>
<tr>
<td><em>P. gingivalis</em></td>
<td>38.1</td>
</tr>
<tr>
<td><em>T. denticola</em></td>
<td>19.1</td>
</tr>
<tr>
<td><em>T. forsythia</em></td>
<td>28.6</td>
</tr>
<tr>
<td><em>F. nucleatum</em></td>
<td>57.1</td>
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Table 3. Multiple logistic regression analysis (stepwise method) of risk of transmission of periodontopathic bacteria from adjacent natural teeth to implants.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Odds Ratio (95%CI)*</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td><em>A. actinomycetemcomitans</em></td>
<td>7.281 (1.39-38.20)</td>
<td>0.0199</td>
</tr>
<tr>
<td><em>P. intermedia</em></td>
<td>4.917 (1.50-16.08)</td>
<td>0.0084</td>
</tr>
<tr>
<td><em>P. gingivalis</em></td>
<td>7.516 (1.72-32.90)</td>
<td>0.0074</td>
</tr>
<tr>
<td><em>T. denticola</em></td>
<td>4.884 (1.27-18.72)</td>
<td>0.0207</td>
</tr>
<tr>
<td><em>T. forsythia</em></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>F. nucleatum</em></td>
<td>3.575 (1.03-12.44)</td>
<td>0.0453</td>
</tr>
</tbody>
</table>

*95% confidence interval
Figure legend

Fig. 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: *A. actinomycetemcomitans*, Pi: *P. intermedia*, Pg: *P. gingivalis*, Td: *T. denticola*, Tf: *T. forsythia*, Fn: *F. nucleatum*
Detection rate (%)

- Natural teeth (N=63)
- Implant (N=21)

Fig. 1