Microbial findings at failing implants


The aim of this study was to evaluate qualitative differences in the subgingival microbiota at titanium implants, ad modum Bränenmark, demonstrating clinical and radiographic signs of loss of supporting tissues (peri-implantitis) as compared to implants surrounded by healthy tissues. A total of 37 patients demonstrating 1 or more implants with bone loss ≥3 threads, bleeding on probing and/or suppuration and 51 patients with clinically healthy mucosa and no bone loss were recruited for the study. In each patient subgingival bacterial samples were obtained using paperpoints, and subjected to microbiological analysis by culture. The two types of clinical conditions showed distinct bacterial profiles. For implants with peri-implantitis putative periodontal pathogens, such as Porphyromonas gingivalis, Prevotella intermedia/Prevotella nigrescens and Actinobacillus actinomycetemcomitans, were found in 60% of the cases and microorganisms primarily not associated with periodontitis, such as Staphylococcus spp., enterics and Candida spp., were found in 55% of the peri-implant lesions. In contrast, implants surrounded by healthy tissue demonstrated a microbiota associated with periodontal health. The results indicate that the microbiota of the healthy peri-implant sulci is similar to that from corresponding conditions around teeth. However, in peri-implant areas staphylococci, enterics and yeasts were found almost as frequently as periopathogens indicating differences as compared to the microbiota around periodontitis affected teeth. A microbiological diagnosis may therefore be of guidance for the choice of antimicrobial treatment in patients with peri-implant infection.


Breakdown of implant supporting tissues does occur, however. According to a recent review (Esposito et al. 1998) on the etiopathogenesis, implant failures are divided into early/late and non-infectious (overload)/infectious (peri-implantitis) failures. A number of factors, e.g., a compromised medical status, smoking, other host-related factors together with operator and biomaterial related factors are associated with increased failure rates which complicates the prediction of the long-term outcome (Esposito et al. 1998). A previous history of periodontitis, i.e., individuals susceptible to periodontal disease and presence of putative periodontal pathogens are other factors that may affect the long-term outcome of an implant treatment. An increased proportion of putative periodontal pathogens has been documented at implant sites of dentate patients as compared to edentulous, suggesting that the periodontal pocket may serve as a reservoir for colonization of titanium implants (Apse et al. 1989; Quirynen &
Listgarten 1990; Danser et al. 1997). Putative periodontal pathogens can be recovered from titanium abutments of partially edentulous patients as early as within 1 month of intraoral exposure (Leonhardt et al. 1993). In breakdown associated with infection (peri-implantitis) a microbiota resembling that of adult periodontitis has been found (Rams et al. 1984, 1991; Mombelli et al. 1987; Becker et al. 1990; Malmstrom et al. 1990; Alcoforado et al. 1991; Rosenberg et al. 1991; Leonhardt et al. 1992). However, microorganisms not primarily associated with periodontitis such as Staphylococcus spp., enterics and Candida spp. have also been isolated (Iacono et al. 1989; Rams et al. 1990; Alcoforado et al. 1991; Slots & Rams 1991). The number of patients involved in the referred studies are, however, too limited for definitive conclusions on specific microbial associations to peri-implantitis.

The objective of this study was to compare the subgingival microbiota, regarding some putative pathogenic microorganisms in areas with clinical and radiographic signs of peri-implantitis to areas adjacent to implants surrounded by clinically healthy mucosa and no radiographic signs of bone loss.

Material and methods

Subjects

Thirty-seven individuals (6 edentulous, 31 dentate), aged 51–79 years (mean age 63 years), with 1–4 failing titanium implants ad modum Bränemark (Nobel Biocare AB, Göteborg, Sweden) were included in the study. These sites demonstrated a progressive marginal bone loss amounting ≥3 threads as compared to 1-year intra-oral radiographs. The peri-implant sulcus was carefully probed and presence of bleeding and/or pus (suppuration) was registered. All implant sites showed a crater-like bony destruction while the apical position of the implant was still osseointegrated. No implant showed mobility. Fifty-one individuals (15 edentulous, 36 dentate), aged 53–79 years (mean age 63 years), without clinical and radiographic signs of disease served as control. All individuals in both groups had 3 or more implants. The duration from the installation were 5 years or more (7±1 years). The teeth were lost due to periodontitis in both edentulous and dentate patients. The periodontal disease was adequately treated before installation and no progression of the attachment levels of the dentate subjects was recorded during the maintenance phase. All individuals were systemically healthy and had not been using any antibiotics during the 2 months prior to the start of the study.

Bacteriological examination

Diseased sites (1–4 sites/patient) and 2–3 sites/patient in the control group were isolated with cotton rolls and supragingival plaque was removed with sterile cotton pellets. Separate subgingival plaque samples were obtained using 3 fine sterile paper points (Johnson and Johnson, East Windsor, NJ, USA). The paper points were inserted to the depth of the peri-implant socket and kept in place for 15 s. The points were transferred to a vial containing 3.3 ml prereduced and anaerobically sterilized VMGA III (Dahlén et al. 1993) and processed in the laboratory within 24 h. After suspension the samples were diluted in VMGA I (Möller 1966) and plated on selective and non-selective media for anaerobic incubation. A volume of 0.1 ml of each dilution was inoculated on Brucella agar plates (BBL Microbiological Systems, Cockeysville, MD, USA) enriched with 5% defibrinated horse blood, 0.5% hemolyzed horse blood, and 5 mg/1 of menadione, and incubated for 7 days in jars using hydrogen combustion in 95% H2 and 5% CO2. Material was also inoculated on TSBV agar plates (Trypticase-soy-agar with 75 mg/ml of Bacitracin and 5 mg/ml of Vancomycin), and incubated for 5 days in air with 10% CO2.

The total viable count (TVC) was determined as the total number of colony forming units obtained on the Brucella agar plates. Species found on selective media were enumerated and their percentage of TVC was calculated. Bacterial colonies were characterized according to its colony and cellular morphology and Gram-stain characteristics, and further identified by biochemical tests. Total counts of Porphyromonas gingivalis and Prevotella intermedia/Prevotella nigrescens were enumerated on the Brucella agar plates. They were identified by their capacity to form black-pigmented colonies. The colonies were also identified by testing UV-fluorescence (Slots & Reynolds 1982) and fermentation of lactose and inodol. Actinobacillus actinomycetemcomitans were enumerated on TSBV agar and tested for catalase reaction. Special attention was paid to growth of enterics and yeast on TSBV agar, and for Staphylococcus spp. on the Brucella agar plate. Enterics were further identified using the API 20E system (API System, Les Balmes de Grottes, Montalieu, France), yeasts by subcultivation on Sabouraud dextrose agar (Difco Laboratories, Detroit, MI, USA) and API 20C system (API System) and Staphylococcus aureus by subculture on Staphylococcus 110 medium (Difco Laboratories) and by being DNAase positive. DNAase negative strains were referred to as Staphylococcus epidermidis. The detection level of the bacterial species was estimated to 1% of TVC,
except for *A. actinomycetemcomitans* for which 100 cells per ml transport medium could be detected.

**Statistics**

The statistical analysis was performed using Fisher’s exact test for comparison of two proportions. Differences between dentate and edentulous patients within the diseased and healthy group were calculated for each bacteria. Differences between the diseased and healthy group were also calculated for each species.

**Results**

A total of 37 patients (6 edentulous and 31 dentate) with peri-implantitis and 51 patients (15 edentulous and 36 dentate) with healthy peri-implant tissues were characterized as shown in Table 1. Both groups had 5 implants at average. In the peri-implantitis group 62% of the individuals were smokers compared to 41% in the healthy group, a difference which did not reach the level of significance. None of the healthy edentulous patients harboured the analysed microorganisms (Table 2), whereas among the healthy dentate patients at least one or more of the analysed species were found in 60% (*P*≤0.001). In diseased edentulous and dentate patients the non-recovery rates were 38% and 10%, respectively. *P. intermedialis*/*P. nigrescens* was the most common group, recovered in 26% of the healthy sulci among dentate patients, in 38% of diseased sites the edentulous patients and in 66% of the peri-implantitis areas among the dentate patients. The difference between the diseased and the healthy group reached statistical significance (*P*<0.01). *A. actinomycetemcomitans* was recovered in 1 edentulous patient and in 31% of the dentate patients of peri-implantitis group. *P. gingivalis* was found in 2 (25%) edentulous and 1 (3%) of the dentate peri-implantitis patients. In the healthy patient group *A. actinomycetemcomitans* and *P. gingivalis* were detected in only 1 patient each. The difference between the diseased and healthy group reached statistical significance for *A.*

<table>
<thead>
<tr>
<th>Table 1. Patient age and sex distribution, number, location and smoking habits in the peri-implantitis and control group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of patients</strong></td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Peri-implantitis group</td>
</tr>
<tr>
<td>Control group</td>
</tr>
</tbody>
</table>

1 One patient with implants in both upper and lower jaw.

<table>
<thead>
<tr>
<th>Table 2. Occurrence (%) of species in dentate and edentulous patients with clinical and roentgenographic signs of disease to the implants as compared to patients with healthy areas adjacent to the implants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microorganisms</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><em>Prevotella intermedia/nigescens</em></td>
</tr>
<tr>
<td><em>Actinobacillus actinomycetemcomitans</em></td>
</tr>
<tr>
<td><em>Porphyromonas gingivalis</em></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
</tr>
<tr>
<td><em>Klebsiella sp.</em></td>
</tr>
<tr>
<td><em>Enterobacter aerogene</em></td>
</tr>
<tr>
<td><em>Enterobacter cloace</em></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
</tr>
<tr>
<td>None of the analysed species</td>
</tr>
</tbody>
</table>

1 *P*<0.05; 2 *P*<0.001.
Leonhardt et al.

actinomycetemcomitans (P<0.01). The other microorganisms investigated were sparse in the healthy sulci but occurred more frequent in peri-implantitis areas (Table 2). None of the patients were found to harbour S. aureus while S. epidermidis (P<0.02) was found in 17% of the diseased dentate group. Enterics was commonly found in the diseased group (30%) compared to the healthy (8%) (P<0.01). Species of Enterobacter and Klebsiella were the most frequently isolated.

Discussion

This study evaluated the prevalence of some microorganisms implicated in the etiopathogenesis of implant failure due to peri-implantitis. The patient selection of the diseased group was based on clinical criteria for peri-implantitis and the high recovery rate of the analysed species in the diseased group confirm the microbial association in peri-implantitis. This does not exclude the involvement of other factors in the etiology or maintenance of the disease. All patients, diseased and healthy, had lost their teeth due to periodontal disease and all dentate patients had undergone periodontal treatment. The peri-implantitis frequency among patients with a periodontal disease history compared to other patients is not known; however, the number of peri-implantitis patients in the present study shows that the condition is not uncommon. Smoking did not come out as a significant factor in this study, however, the higher frequency of smokers in the peri-implantitis group implicate that smoking could be an important factor.

The present study demonstrated a different microbiota between the healthy and the diseased peri-implant sulci. Both groups showed certain similarities to that of corresponding conditions around teeth which is in accordance with earlier studies (Rams & Link, 1983; Rams et al. 1984, 1991; Mombelli et al. 1987; Becker et al. 1990; Leonhardt et al. 1992). The presence of putative periodontal pathogens around teeth does not necessarily lead to periodontal tissue breakdown; however, presence of P. gingivalis, A. actinomycetemcomitans and Bacteroides forsythus confers an increased risk for microbiologically induced periodontal breakdown (Beck et al. 1990; Haffajee et al. 1991; Brown et al. 1994; Grossi et al. 1995). It is generally anticipated that elevated numbers of these bacteria ought to be present for extended periods of time in order to have an impact on the periodontal tissues (Socransky et al. 1982). Determining whether colonization of implants by putative periodontal pathogens in fact entails an increased risk for microbiologically induced peri-implant tissue breakdown is not within the scope of this study. It is however interesting to note that 60% of the patients with peri-implantitis harboured periodontal pathogens, such as P. gingivalis, P. intermedia/P. nigrescens and A. actinomycetemcomitans. This is in accordance with previous reports (Augthun & Conrads, 1997; Becker et al. 1990; Mombelli et al. 1987; Saleetti et al. 1997) and thus confirms that they constitute indicators for a diseased condition. Interestingly P. gingivalis and A. actinomycetemcomitans were found not only in dentate but also in edentulous patients. Danser et al. (1997) suggested that elimination of the subgingival environment by extraction of all teeth probably initiates the disappearance of these two bacterial species. This study shows that this might not be true due to the fact that late (>5 years) occurring peri-implantitis in edentulous patients harbour a microbiota which resembles the one at dentate individuals.

The major finding in the present study was the frequent recovery of microorganisms which are associated with a medical compromised status. Although coagulase-negative staphylococci could frequently be found in saliva, they are usually low in proportions and less frequent in plaque (Percival et al. 1991). S. aureus is infrequently found in the oral cavity and often in connection with pathology (Öhman et al. 1995). The prevalence of oral staphylococci are increased with age and denture wearing (Marsh et al. 1992). Both S. aureus and coagulase negative species are the most common microorganisms in association with indwelling medical device infections in general (Bisno & Waldvogel, 1989; Clarke & Raffin 1990). S. aureus is even suggested to be the most common bacteria responsible for infections associated with metallic biomaterials (Green & Ripley 1984; Gristina 1987). In a study by Brause (1989) S. aureus and S. epidermidis were found to be the major pathogens at infected orthopaedic prosthesis. Staphylococci has gained interest also in periodontal disease (Rams et al. 1990; Dahlén & Wikström 1995); however, their etiological relationship has not yet been clarified. Interestingly, peri-implantitis lesions exhibited significantly higher proportions of staphylococci than periodontitis lesions (Rams et al. 1990; Iacono et al. 1989; Alcoforado et al. 1991; Slots & Rams 1991). This was confirmed in the present study, especially in those individuals who were partially edentulous.

Similarly to staphylococci yeasts could be isolated infrequently and in low amounts in the average person's mouth (Percival et al. 1991). They increase even more pronounced than staphylococci with age and denture wearing (Marsh et al. 1992). Also in association with periodontitis yeasts could infrequently be present, however in low amounts...
Microbial findings at failing implants

(Slots et al. 1988; Dahlén & Wikström 1995). Due to the preference of yeasts for an aerobic environment, their etiological relationship to periodontitis could be questioned. Similarly, the significance of yeasts in peri-implantitis lesions could be questioned. Also in the present study yeasts were only sporadically present in the peri-implantitis lesions and preferentially in the partially edentulous cases.

Enterics such as Escherichia coli, Enterobacter spp., Klebsiella spp. and Pseudomonas are frequently found in the oral cavity (Chang & Foltz 1960; Dahlén et al. 1982; Köndell 1984; Sanchez-Cordero et al. 1979). These microorganisms are mainly referred to as the transient part of the oral microbiota. Commonly, these bacteria have been associated with mucosal infections in medically compromised patients, in patients with dentures and in elderly patients (Bergman, 1988; Dahlén et al. 1982; Klästersky 1985). They have also been recognized in patients with periodontal disease and in proportions frequently more than 10% of the TVC (Rams et al. 1990, 1992; Slots et al. 1988, 1990a). This might indicate an etiological role of enterics in some periodontitis cases. From the present study it can be further concluded the enterics also, for the same reason, may play a significant role in peri-implantitis lesions. Therefore, it is important to note that opportunistic microorganisms are found almost as frequently as anaerobic periopathogens in peri-implant lesions. Some of these species are quite resistant to various forms of antimicrobial treatment, antiseptics as well as antibiotics (Slots et al. 1990b, 1991). The microbial diversity found in the present study, should therefore be considered in the treatment strategy of peri-implantitis.

Acknowledgements
This study was supported by grants from the Research fund, Botus County and by Oral Microbiological Diagnostic Service, Göteborg University, Sweden.

Résumé
Le but de cet étude a été d’évaluer les différences qualitatives entre la flore sous-gingivale d’implants en titane ad modum Bränemark qui avaient des signes cliniques et radiographiques de perte des tissus de soutien (paro-implantite) et celle associée à des implants entourés de tissus sains. Trente-sept patients ayant un ou plusieurs implants avec une perte osseuse ≥ 3 filetages, saignement au sondage et/ou supputation, et cinquante et un patients avec une muqueuse cliniquement saine et sans perte osseuse ont participé à cette étude. Chez chaque patient des échantillons bactériens sous-gingivaux ont été obtenus à l’aide de pointes de papier et soumis à l’analyse microbiologique par culture. Les deux types de conditions cliniques avaient des profils bactériens différents. Au niveau des implants avec des pathogènes parodontaux putatifs de la paro-implantite tels que le Prophyromonas gingivalis, les Prevotella intermedia/Prevotella nigrescens et l’Actinobacillus actinomycetemcomitans, ces derniers ont été trouvés dans 60% des cas tandis que des microorganismes essentiellement non-associés avec la parodontite tels que les Staphylococcus spp., les entérobactéries et les Candida spp. ont été trouvés dans 55% des lésions paro-implantaires. Par contre, les implants entourés de tissus sains démontraient une microflore associée avec un parodontite sain. Les résultats indiquent que la microflore autour d’un implant sain est semblable à celle qui se retrouve autour des dents. Cependant, dans les zones paro-implantaires des staphylocoques, des entérobactéries et des levures étaient trouvés quasi aussi fréquemment que les paropathogènes mettant en évidence des différences lorsque la comparaison était faite avec la microflore associée à la parodontite. Un diagnostic microbiologique pourrait donc être un guide dans le choix du traitement antimicrobien chez les patients avec paro-implantite.

Resumen
La intención de este estudio fue evaluar las diferencias cualitativas en la microbiología subgingival en los implantes de titanio, del tipo Bränemark, que demostraron unos signos clínicos y radiográficos de pérdida de tejidos de soporte (periimplantitis) en comparación con implantes rodeados de tejidos sanos. Se reclutaron para este estudio a 37 pacientes que demostraron uno o más implantes con una pérdida ósea ≥3, roscas, sangrado al sondaje y/o supuración y 51 pacientes con mucosa clínicamente sana sin pérdida ósea. En cada paciente se obtuvieron muestras bacterianas subgingivales usando puntos de papel, sometiéndose a análisis por cultivo. Las dos condiciones clínicas mostraron diferentes perfiles bacterianos. Para los implantes con periimplantitis se encontraron en un 60% de los casos patógenos periodontales putativos, tales como Porphyromonas gingivalis, Prevotella intermedia/Prevotella nigrescens y Actinobaci-
References


