Clin Oral Invest (2012) 16:843–850
DOI 10.1007/s00784-011-0561-8

ORIGINAL ARTICLE

Metagenomic analysis of the peri-implant and periodontal microflora in patients with clinical signs of gingivitis or mucositis

Wieland Heuer · Andreas Kettenring · Sascha Nico Stumpp · Jörg Eberhard · Eva Gellermann · Andreas Winkel · Meike Stiesch

Received: 22 July 2010 / Accepted: 18 April 2011 / Published online: 3 May 2011

Abstract The long-term success of osseointegrated oral implants is endangered by inflammation of peri-implant hard and soft tissues caused by bacterial biofilms that may have been initiated by bacterial transmission from the adjacent dentition. The present study aimed to compare the bacterial communities at inflamed implant and tooth sites by broad-range PCR techniques to evaluate the etiological processes of peri-implant and periodontal diseases and potential future therapeutic strategies. Eighteen samples of peri-implant and periodontal microflora were collected from nine partially edentulous patients with implant-retained crowns or bridges revealing clinical signs of gingivitis or mucositis. The clinical parameters plaque index (PI), probing depth (PD), and bleeding on probing were recorded. Amplified fragments of bacterial 16S rRNA genes were separated by use of single-strand conformation polymorphism analysis, and sequences were determined to identify the predominant bacterial genera. The clinical parameters PI and PD were significantly different at implants (PI=0.4±0.7, PD=3.1±0.6 mm) compared with teeth (PI=1.8±0.8, PD=2.5±0.2 mm). A total of 20 different genera were found at the inflamed tooth and implant sites. The microbial diversity of the microflora surrounding the remaining dentition (12.0±3.8) was significantly higher (p=0.01) than the diversity of the peri-implant microflora at implant-retained crowns or bridges (6.3±2.3). Within the limitations of the present study, the microbial diversity of the investigated implants and teeth with clinical signs of mucositis or gingivitis exhibits substantial differences, demonstrating that transmission of the complete bacterial microflora from teeth to implants could be excluded. Furthermore, broad-range molecular biological detection methods specify bacterial genera and species in the peri-implant and periodontal microflora which were not in the focus of research interests so far.

Keywords Bacterial biofilms · Dental implants · Microbial diversity · Single-strand conformation polymorphism

Introduction

The clinical success of osseointegrated oral implants has encouraged their increased use, and many conventional prosthetic treatments have been replaced by implant-retained prostheses. While the problem of primary osseointegration has been convincingly solved, inflammation of peri-implant hard and soft tissues caused by bacterial biofilms is now regarded as one of the principal problems in dental implantation with the highest incidence of implant loss within the first 12 months [1–3]. The early processes of supra- and subgingival biofilm formation, such as the generation of an acquired pellicle from salivary biopolymers or enzymes and the adherence of early colonizing micro-organisms, have been described together with the relationship between biofilm formation and periodontitis or peri-implantitis,

Wieland Heuer and Andreas Kettenring contributed equally to the manuscript.

W. Heuer (✉) · S. N. Stumpp · J. Eberhard · E. Gellermann · A. Winkel · M. Stiesch
Department of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School,
Carl-Neuberg-Strasse 1,
30625 Hannover, Germany
e-mail: Heuer.Wieland@mh-hannover.de

A. Kettenring
Salzgitter, Germany

respectively [4]. For example, *Streptococcus* or *Actinomyces* species are known to create the preconditions for the accumulation of Gram-negative anaerobic late-colonizing microorganisms, such as *Fusobacterium* or *Prevotella* species [5–11]. These bacteria, as well as *Aggregatibacter actinomycetemcomitans* or *Porphyromonas gingivalis*, have been frequently isolated from diseased periodontal or peri-implant sites and have been designated as highly relevant for the development of chronic periodontal or peri-implant inflammatory processes [12]. Several studies have shown that the microbial composition shifts toward a higher proportion of periodontal pathogens during peri-implant biofilm formation [12–15]. This shift, as well as the general process of biofilm formation, is affected by tongue activity and ecological cofactors like pocket formation, salivary composition, and nutrition [16–19]. The contribution of different implant surface characteristics to the accumulation of biofilms and the following clinical consequences are controversially discussed [20].

The pathological processes as well as the bacterial flora at implants and periodontitis-affected teeth have been described in detail, supporting the hypothesis that a cross-contamination from the dentition to implants takes place, endangering non-inflamed conditions at implant sites [21]. However, gingivitis has a prevalence 20-fold the prevalence of periodontitis in western population, indicating a status of inflammation with high loads of pathogenic bacteria [22]. Gingivitis is a substantial precursor for periodontitis and may also contribute to the development of mucositis and peri-implantitis. Several different strategies have been used to identify potential pathogens, and recently, 16S rRNA gene-based techniques have been added to this repertoire, which have the advantage to potentially detect the complete genome of a bacterial community irrespective of any known bacteria. The SSCP analysis method in combination with sequencing was successfully used for the detection of the microflora at implants and teeth [20, 23]. Therefore, the aim of the present study was to compare for the first time the microbial diversity of peri-implant and periodontal microflora in partially edentulous patients revealing clinical signs of gingivitis or mucositis by use of a broad-range molecular biological detection method to test the hypothesis of similar bacterial communities at implant and tooth sites that may reveal the extent of bacterial transmission from teeth to implants in a situation of disease.

Method and materials

Patients

This study was approved by the ethics committee of Hannover Medical School (no. 3791). The examination was performed with the understanding and written consent of each patient.

The qualitative and quantitative analysis was based on partially edentulous patients with crowns or bridges cemented on implant abutments. All patients were treated with at least one oral two-piece implant made of titanium without any individual surface modification (Tissue Level, Institut Straumann, Switzerland) in the upper or lower jaw between 2005 and 2006 and an implant loading 3 months after a one-step surgery. After a minimum of 2 years of function within the oral cavity and routine oral home care procedures and preventive appointments in a dental clinic, the patients were selected to fulfill the following inclusion criteria: no systemic disease such as diabetes mellitus, no smoking during or up to 12 months before the start of the study, and no pregnancy. The patients did not show any history of periodontitis or radiographic bone loss >3 mm and no probing pocket depth at implants or teeth ≥4 mm. At implants and dentition, the tissues showed manifest signs of inflammation, like redness and swelling as well as bleeding on probing, and were diagnosed as gingivitis or mucositis. No pharmacological treatment or antibiotic therapy was reported during or up to 4 months before the recordings.

Five women and four men (aged between 27 and 66 years, mean 50±13 years) qualified for the following procedures.

Bacterial samples were taken at four sites for each implant and the respective tooth in the same jaw. The sampling area was isolated from saliva, gently dried by air, and the supragingival plaque was not removed. Four paper points were inserted for 10 s into the peri-implant or gingival sulcus (mesio-buccal, disto-buccal, mesio-palatal/lingual, disto-palatal/lingual) and pooled for every implant or tooth. All samples were stored in Eppendorf tubes (Eppendorf, Hamburg, Germany) at −80°C before processing.

Periodontal and peri-implant examination

Probing depth and bleeding on probing were obtained at six different sites (mesio-buccal, buccal, disto-buccal, mesio-oral, oral, disto-oral) per Ramfjord teeth and implant, and the plaque index (Silness and Loe) was determined at four sites (mesio-buccal, disto-buccal, mesio-oral, and disto-oral) per Ramfjord teeth. All clinical examinations were carried out by the same trained clinician using a marked periodontal probe (WHO-DMS probe, Deppeler, Rolle, Switzerland). The probing depth was measured to the nearest millimeter on the scale.

Comparison of the clinical data was performed using a two-tailed Wilcoxon test for paired, non-normally distributed data. The level of significance was set to $p \leq 0.05$.

Nucleic acid extraction

Total genomic DNA was isolated using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany). Preparation was

according to the manufacturer's protocol for bacteria, with an additional mechanical disruption step for complete lysis of gram-positive and gram-negative species. For this purpose, samples were treated with 20 mg/ml hen egg white lysozyme (Fluka, Buchs, Switzerland) for 30 min at 37°C in lysis buffer (20 mM Tris–HCl, 2 mM EDTA, 1.2% Triton X100, pH 8.00), followed by proteinase K digestion. The cell suspension was homogenized (6,500 rpm, 3×20 s, 15-s break) with a Precellys 24 bead mill (Bertin Technologies, Montigny-le-Bretonneux, France) using 0.5-mm glass beads (Roth, Karlsruhe, Germany). Isolated DNA was stored at −20°C.

Amplification of the 16S rDNA and exonuclease digestion

An approximately 500-bp fragment of the 16S rRNA gene was amplified using the universal primers 27f (5′-AGAGTTTGATCMTGGCTCAG-3′; MWG Biotech, Ebersberg, Germany) and 5′-phosphorylated 521revP (5′-ACCGCGGCTGCTGGCAC-3′, MWG Biotech). These primers target conserved regions flanking the V1 and V3 hypervariable regions within the 16S rRNA gene. The polymerase chain reaction (PCR) was performed on a TProfessional thermocycler (Biometra, Göttingen, Germany). The PCR mix contained 50 ng of template DNA, 200 nM of each primer, 1× PCR buffer (including 1.5 mM magnesium chloride, Qiagen), 1.5 U HotStarTaq polymerase (Qiagen), 200 mM of each dNTP (Roth), and PCR grade water (Roche, Penzberg, Germany) in a total reaction volume of 50 μl. PCR conditions were as follows: initial denaturation at 95°C for 15 min; 30 amplification cycles consisting of denaturation at 94°C for 1 min, annealing at 52°C for 40 s, elongation at 72°C for 1 min; and final extension at 72°C for 10 min. A total volume of 5 μl of each amplification reaction was analyzed by agarose gel electrophoresis (Agarose MP, AppliChem, Darmstadt, Germany). PCR products were purified using the QIAquick PCR Purification kit (Qiagen). Single-stranded DNA (ssDNA) was generated by enzymatic cleavage. For this purpose, 1.5 μg of each PCR product was digested with 10 u lambda exonuclease (NEB, Frankfurt am Main, Germany) in 1× exonuclease buffer (NEB) for 1 h at 37°C in a total volume of 55 μl. The enzymatic reaction products were purified using the QIAquick PCR purification kit (Qiagen) and the samples dried overnight in a thermal shaker (40°C, 800 rpm; Thermomixer comfort, Eppendorf) and subsequently stored at −20°C until further processing.

Sequence-dependent separation of 16S rDNA fragments

Single-strand conformation polymorphism (SSCP) analyses were performed on a DCode Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA). For this purpose, ssDNA fragments were resuspended in 5 μl 1× SSCP buffer (Bio-Rad), heated for 5 min to 95°C, and kept on ice for 3 min prior to electrophoresis. Subsequently, samples were loaded on a 0.625× MDE gel (Lonza, Rockland, ME, USA). Electrophoresis was performed at 300 V (20°C) for 24 h in 0.7× TBE buffer (Bio-Rad). DNA bands were visualized by silver staining according to the manufacturer's instructions (Silver-Stain kit, Bio-Rad).

Band extraction, re-amplification, and sequencing

Bands were excised from the gel, homogenized, and resuspended in 100 μl elution buffer (0.5 M ammonium acetate, 10 mM magnesium acetate, 1 mM EDTA, 0.1% sodium dodecyl sulfate, pH 8.0). DNA was eluted overnight on a thermal shaker (50°C, 800 rpm; Thermomixer comfort, Eppendorf). Samples were concentrated by ethanol precipitation and resuspended in 10 μl double distilled water. The complete DNA solution was used as template for PCR re-amplification with the primer pair 27f/521revP. The PCR conditions were the same as described above. However, the cycle number was increased to 33 and the annealing temperature was raised to 54°C. Afterwards, PCR products were purified using the QIAquick MinElute kit (Qiagen) and were subsequently sequenced (Seqlab, Göttingen, Germany). The obtained sequences were checked using the BioEdit software package (v7.0.9, Ibis Biosciences, Carlsbad, CA, USA) and compared with the nucleotide sequence database from the National Center for Biotechnology Information. For identification of the closest match, both the BLAST Basic Local Alignment Search Tool and the SEQMATCH Tool from the Ribosome Database Project were used [24, 25].

Counting the SSCP profiles and statistical analysis

The 16S rDNA banding pattern of each sample was analyzed using the Quantity One 1D-Analysis Software package (v4.6.5, Bio-Rad). The total number of bands was determined after background subtraction (rolling circle correction; disc size, 30) with preset values for sensitivity of 5.1 and a minimal band intensity of ≥2% [20, 26].

The analysis compared the microbial diversity of the peri-implant microflora compared with the remaining dentition, and the null hypothesis is rejected if a significant difference is detected between implant-retained crowns or bridges and the remaining dentition.

The null hypothesis is:

- $H_{0\ (1)}$: No difference between implant-retained crowns or bridges and the remaining dentition in microbial diversity
- $H_{A\ (1)}$: Significant difference between implant-retained crowns or bridges and the remaining dentition in microbial diversity

A post hoc power calculation using the results of the quantitative SSCP analysis (mean1±SD1=12.0±3.8, mean2±SD2=6.3±2.3, standard deviation of the differences=3.8) revealed that a sample size of 9 has 94% power to detect a statistical difference. Power and sample sizes were calculated using nQuery Advisor 5.0 (Statistical Solutions, Saugas, MA, USA). Comparison of the data was performed using a two-tailed Wilcoxon test for paired, non-normally distributed data. The level of significance was set to $p \leq 0.05$. Documentation and evaluation of the data was performed with the data processing program SPSS/PC version 18.0 for Windows (SPSS, Chicago, IL, USA).

Results

Clinical examination

The results of the sulcular and peri-implant examination are summarized in Table 1. The plaque index of the observed dentition was 1.8±0.8, which was significantly higher compared with the implant-retained crowns or bridges (0.4±0.7, $p=0.014$). The mean probing depth measurements at the peri-implant sites were 3.1±0.6 mm, which was significantly higher than probing depth measurements at the observed teeth (2.5±0.2 mm, $p=0.008$). Bleeding on probing values were not significantly different ($p=0.260$) between implants (58±28%) and the observed teeth (43±28%).

Sequence-dependent separation of 16S rDNA fragments

For the evaluation of the microbial diversity, the amplified bacterial 16S rDNA was separated by SSCP. Figure 1 summarizes the number of predominant SSCP gel bands at implants compared with the observed teeth in partially edentulous patients. Samples from the gingival sulcus exhibited 12.0±3.8 predominant bands per lane, which was significantly higher than the diversity of the peri-implant microflora (6.3±2.3 bands per lane, $p \leq 0.01$).

Band extraction, re-amplification, and sequencing

To identify the most abundant bacterial genera in the crevicular fluid, the bands of the SSCP fingerprints were excised and the polynucleotide sequences of the fragments were determined. A total of 20 different genera were found at both sites, whereas 19 different sequences were found at teeth and 6 at implants. The most frequent genera were *Fusobacterium*, *Prevotella*, *Porphyromonas*, *Streptococcus*, *Campylobacter*, and *Neisseria* (Table 2). Twelve bacterial genera like *Neisseria* or *Campylobacter* were not found at implant sites, but were frequently isolated at dental sites. For example, patient 5 exhibited the bacterial genera *Prevotella*, *Leptotrichia*, *Capnocytophaga*, *Campylobacter*, and *Paludibacter* at tooth sites, but not at implant sites. In contrast, members of the candidate division TM7 were detected solely at implant sites. Table 2 also demonstrated that various bacterial genera were found at the observed tooth sites, in contrast to only a few different genera found at implant sites.

Table 1 Plaque index, probing depth, and bleeding on probing at implant and tooth sites (mean and standard deviation)

	Plaque index	Probing depth	Bleeding on probing (%)
Observed teeth	1.8±0.8	2.5±0.2	43±28
Implants	0.4±0.7	3.1±0.6	58±28

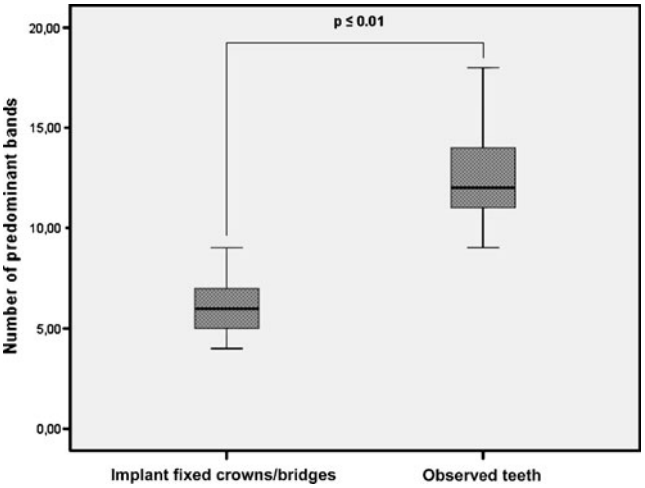

Fig. 1 Number of predominant SSCP gel bands at implants ($n=9$) compared with the observed teeth ($n=9$) in partially edentulous patients

Discussion

Within the limits of the present study focused on one implant system, the results demonstrated for the first time (a) the bacterial diversity of the sulcular flora at inflamed tissues of implants and teeth using broad-range PCR techniques, (b) the high bacterial diversity of natural teeth compared with implants, and (c) different bacterial compositions at implant and teeth habitats in the same individual.

Bacterial colonization of dental implants may be followed by chronic inflammation of peri-implant hard and soft tissues. This bacterial-induced inflammation is considered to be one of the main challenges in dental implantation and is the main cause of early implant failure. Several studies have demonstrated that the long-term prognosis of osseointegrated implants depends on the

Table 2 Bacterial genera at implants and the observed teeth in partially edentulous patients (1–9)

	Observed teeth									Implants								
Patient no.	9	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8	9
Fusobacterium	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓		✓	✓			✓
Prevotella			✓	✓	✓			✓	✓	✓	✓	✓	✓					
Porphyromonas		✓			✓		✓	✓						✓				✓
Streptococcus	✓		✓	✓		✓									✓			
Veillonella	✓		✓						✓						✓			
Campylobacter			✓		✓	✓			✓									
Neisseria	✓	✓	✓					✓										
Haemophilus									✓	✓					✓			
Lactobacillus	✓					✓	✓											
Rothia	✓	✓						✓										
Paludibacter		✓	✓		✓													
Gemella	✓		✓															
Capnocytophaga					✓			✓										
Leptotrichia					✓													
Aggregatibacter									✓									
Moraxella						✓												
Eikenella				✓														
Selenomonas		✓																
Actinomyces				✓														
TM7 genera incertae sedis																	✓	

biofilm mass and the colonizing species within the biofilm [15, 27–29]. For this reason, the analysis of yet unknown bacterial genera is of great relevance for preventive and therapeutic strategies of peri-implant infections. However, until now, the microbial diversity around implants and teeth was analyzed by bacterial culture or species-specific detection methods like DNA–DNA hybridization or PCR [30, 31]. The use of these methods revealed no differences between the microbial community of periodontal and peri-implant microflora of severely inflamed and non-inflamed tissues [21, 32] and may lead to the assumption that implants were colonized by bacteria located on residual teeth. This assumption was supported by Danser et al. [33] who were able to indicate the crucial role of periodontal pockets for the transmission of periopathogens within the oral cavity. In contrast, the present study demonstrated that the diversity at tooth surfaces is more complex than at implant sites and that several tooth sites were contaminated with bacterial genera that were not present at implants. This observation does not only negotiate the existence of cross-contamination but also demonstrates the establishment of an implant-specific bacterial flora that is different from that of the colonizing teeth in the same individual.

It is of relevance that the study included implants and teeth showing signs of inflammation, which demonstrated the pathological capacities of both bacterial communities. It is likely that the bacterial composition is a consequence of the different surfaces at implant and tooth sites, the anatomical specifics of the mucosal or gingival tissues, and the diverse inflammatory reactions.

Separation of the bacterial 16S rDNA amplicons was performed by use of the SSCP method. This highly sensitive procedure in combination with the subsequent sequence analysis affords detection and identification of predominant bacteria in the peri-implant and dental microflora. Thus, the applied technique avoids the main disadvantage of the conventional PCR and DNA hybridization methods where only the expected bacteria can be detected by use of specific primers. Moreover, the detection and identification of oral bacteria on the basis of 16S rDNA fingerprints avoids time-consuming and fault-prone bacterial cultivation techniques [34] because the detection does not depend on the viability of the bacteria. In addition to chemical disruption, an additional mechanical disruption step was used to reveal bacterial DNA with high efficiency. Irrespectively, of the noted advantages of broad-range sequencing techniques, a main limitation is the lower detection limit compared with conventional PCR techniques. The use of species-specific primers enables a more specific detection of bacteria in minimal numbers, which may be one reason why no bacteria were found at implant no. 7.

In a study of Keller et al. [35], microbial examination of implant-cemented crowns was performed using bacterial cell cultures. They found members of the genus *Fusobacterium* as the most abundant bacterial species in the peri-implant region of cemented crowns, which is in accordance with the present work. In both studies, *Prevotella* was also detected as the second most abundant bacterial genus in the peri-implant microflora. In comparison to the present study, this research group identified 14 different bacterial species in the remaining dentition (probing depth≤3 mm) and seven predominant microbial species in the peri-implant crevicular fluid (probing depth≤3 mm). The recorded microbial diversities were slightly smaller, although this might be explained by the detection of only living bacteria using bacterial cultivation techniques.

As the present study employs a highly sensitive molecular biological SSCP method for the detection of all predominant bacterial genera, the results provide the first evidence of more bacterial genera in the sulcus fluid of natural teeth with signs of gingivitis than in the crevicular fluid of dental implants with clinical signs of mucositis in the same patient. The data of the present study have several implications for future clinical research and therapeutic strategies. First, any antimicrobial therapy for peri-implant diseases have to take into account a specific bacterial diversity different from the microbiota in periodontal diseases. Second, other oral niches than the natural teeth have to be considered as a reservoir for bacteria inducing peri-implant diseases. Third, the disease progression, inflammatory processes, and therapeutic strategies may be different for peri-implantitis compared with periodontitis due to the differences in microbiology.

The attendance of some bacterial genera in the peri-implant crevicular fluid does not necessarily imply that these bacterial genera could be detected also in the periodontal microflora. For example, members of the candidate division TM7 were found in the peri-implant microflora, but not in the periodontal microflora of the same patient. TM7 was indicated as a member of the oral microbiome earlier and is referenced in the Oral Microbiome database [36].

For the present study, the supragingival plaque was not removed prior to sampling because the comprehensive biofilm sample adjacent to the mucosa or marginal gingival participated in the development of the mucosal or gingival lesions investigated. The area was dried before sampling to avoid any contamination from bacteria which were floating in the saliva and were not part of the microbiota of the established and attached biofilms investigated in the present study, although it is acknowledged that saliva is a bacterial reservoir for biofilm growth. Explanations for the development of different microbial communities at implant and tooth sites in the present study include diverse biofilm formation on artificial implant surfaces compared with naturally tooth hard substances, different immune capacities of the peri-implant tissues compared with the cells of the epithelial sulcus, and different environments at peri-implant and sulcular sites. The results of several studies indicated the contribution of material characteristics on initial events during oral biofilm formation [37, 38], and the effects of surface characteristics like roughness and surface free energy for the microbial composition at implants or natural teeth are obvious. In the present study, probing depth measurements were significantly different between implant and tooth sites, which are likely a consequence of different adhesion mechanisms between implants and mucosa, respectively teeth and gingiva. However, lower probing depths do not necessarily imply lower peri-implant microbial diversity because the different ecosystems are not only influenced by the oxygen gradients within the peri-implant or periodontal pockets but also by the mass of oral biofilms and the oxygen gradients within the biofilms. Preza et al. [39] analyzed the diversity and site specificity of the oral microflora in the elderly by use of a 16S rRNA gene-based microarray method. Similar to the present study, they showed that the bacterial flora appears site-specific for different oral niches and subject-specific bacterial profiles were not evident. These results are in accordance with the authors' hypothesis that all-embracing cross-infection loses evidence by extending the detection methods on more bacterial genera or species. However, the different objectives of the studies have to be considered carefully while interpreting similar results of the two studies. In accordance with these observation, Lindhe et al. [40] reported pronounced clinical and radiographic signs of tissue destruction at implants compared with teeth following subgingival plaque formation. This result supports the assumption that peri-implant tissues do not have the same potential to combat pathogenic microbiota, thus resulting in different predominant bacterial genera or species. In addition, the significant difference of the plaque index at the observed teeth and the implant-retained crowns or bridges plays a decisive role for the development of a periodontal and peri-implant microflora with many different predominant bacterial genera.

In summary, the present study demonstrates for the first time that the bacterial diversity of implants and teeth in patients with clinical signs of gingivitis or mucositis exhibits substantial differences. Based on the observation that the bacterial flora of teeth and implants are different, transmission of the complete bacterial microflora from teeth to implants could be excluded.

Acknowledgments We are indebted to Ralph Scherer and Dr. Ludwig Hoy from the Institute for Biometry at the Hannover Medical School for the excellent statistical consultancy. This study was supported by the Deutsche Forschungsgemeinschaft (SFB 599 TP D8; PI: M. Stiesch)

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Snauwaert K, Duyck J, van Steenberghe D, Quirynen M, Naert I (2000) Time dependent failure rate and marginal bone loss of implant supported prostheses: a 15-year follow-up study. Clin Oral Investig 4(1):13–20
2. Esposito M, Hirsch JM, Lekholm U, Thomsen P (1998) Biological factors contributing to failures of osseointegrated oral implants. (II). Etiopathogenesis. Eur J Oral Sci 106(3):721–764
3. Berglundh T, Persson L, Klinge B (2002) A systematic review of the incidence of biological and technical complications in implant dentistry reported in prospective longitudinal studies of at least 5 years. J Clin Periodontol 29(Suppl 3):197–212, discussion 232–193
4. Heuer W, Elter C, Demling A, Neumann A, Suerbaum S, Hannig M, Heidenblut T, Bach FW, Stiesch-Scholz M (2007) Analysis of early biofilm formation on oral implants in man. J Oral Rehabil 34 (5):377–382
5. Slots J (1977) The predominant cultivable microflora of advanced periodontitis. Scand J Dent Res 85(2):114–121
6. Loe H, Theilade E, Jensen SB (1965) Experimental gingivitis in man. J Periodontol 36:177–187
7. Quirynen M, Vogels R (2002) Clinical relevance of surface characteristics on the formation of plaque on teeth and implants. Ned Tijdschr Tandheelkd 109(11):422–429
8. Slots J (1977) Microflora in the healthy gingival sulcus in man. Scand J Dent Res 85(4):247–254
9. Hannig M (1997) Transmission electron microscopic study of in vivo pellicle formation on dental restorative materials. Eur J Oral Sci 105(5 Pt 1):422–433
10. Li J, Helmerhorst EJ, Leone CW, Troxler RF, Yaskell T, Haffajee AD, Socransky SS, Oppenheim FG (2004) Identification of early microbial colonizers in human dental biofilm. J Appl Microbiol 97(6):1311–1318
11. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE (2005) Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 43(11):5721–5732
12. Leonhardt A, Berglundh T, Ericsson I, Dahlen G (1992) Putative periodontal pathogens on titanium implants and teeth in experimental gingivitis and periodontitis in beagle dogs. Clin Oral Implants Res 3(3):112–119
13. Berglundh T, Lindhe J, Marinello C, Ericsson I, Liljenberg B (1992) Soft tissue reaction to de novo plaque formation on implants and teeth. An experimental study in the dog. Clin Oral Implants Res 3(1):1–8
14. Ericsson I, Berglundh T, Marinello C, Liljenberg B, Lindhe J (1992) Long-standing plaque and gingivitis at implants and teeth in the dog. Clin Oral Implants Res 3(3):99–103
15. Abrahamsson I, Berglundh T, Lindhe J (1998) Soft tissue response to plaque formation at different implant systems. A comparative study in the dog. Clin Oral Implants Res 9(2):73–79
16. Rozen R, Bachrach G, Steinberg D (2004) Effect of carbohydrates on fructosyltransferase expression and distribution in *Streptococcus mutans* GS-5 biofilms. Carbohydr Res 339(18):2883–2888
17. Lima EM, Koo H, Vacca Smith AM, Rosalen PL, Del Bel Cury AA (2008) Adsorption of salivary and serum proteins, and bacterial adherence on titanium and zirconia ceramic surfaces. Clin Oral Implants Res 19(8):780–785
18. Kreth J, Merritt J, Qi F (2009) Bacterial and host interactions of oral streptococci. DNA Cell Biol 28(8):397–403
19. Demling A, Demling C, Schwestka-Polly R, Stiesch M, Heuer W (2009) Influence of lingual orthodontic therapy on microbial parameters and periodontal status in adults. Eur J Orthod 31:638–642
20. Grossner-Schreiber B, Teichmann J, Hannig M, Dorfer C, Wenderoth DF, Ott SJ (2009) Modified implant surfaces show different biofilm compositions under in vivo conditions. Clin Oral Implants Res 20(8):817–826
21. Gouvoussis J, Sindhusake D, Yeung S (1997) Cross-infection from periodontitis sites to failing implant sites in the same mouth. Int J Oral Maxillofac Implants 12(5):666–673
22. Brown LJ, Johns BA, Wall TP (2002) The economics of periodontal diseases. Periodontol 2000 29:223–234
23. Heuer W, Stiesch M, Abraham WR (2011) Microbial diversity of supra- and subgingival biofilms on freshly colonized titanium implant abutments in the human mouth. Eur J Clin Microbiol Infect Dis 30:193–200
24. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215(3):403–410
25. Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM, Tiedje JM (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 37(Database issue):D141–D145
26. Seksik P, Rigottier-Gois L, Gramet G, Sutren M, Pochart P, Marteau P, Jian R, Dore J (2003) Alterations of the dominant faecal bacterial groups in patients with Crohn's disease of the colon. Gut 52(2):237–242
27. Mombelli A, van Oosten MA, Schurch E Jr, Land NP (1987) The microbiota associated with successful or failing osseointegrated titanium implants. Oral Microbiol Immunol 2(4):145–151
28. Ong ES, Newman HN, Wilson M, Bulman JS (1992) The occurrence of periodontitis-related microorganisms in relation to titanium implants. J Periodontol 63(3):200–205
29. Kolenbrander PE (2000) Oral microbial communities: biofilms, interactions, and genetic systems. Annu Rev Microbiol 54:413–437
30. Salvi GE, Furst MM, Lang NP, Persson GR (2008) One-year bacterial colonization patterns of *Staphylococcus aureus* and other bacteria at implants and adjacent teeth. Clin Oral Implants Res 19 (3):242–248
31. Mineoka T, Awano S, Rikimaru T, Kurata H, Yoshida A, Ansai T, Takehara T (2008) Site-specific development of periodontal disease is associated with increased levels of *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* in subgingival plaque. J Periodontol 79(4):670–676
32. Quirynen M, Vogels R, Peeters W, van Steenberghe D, Naert I, Haffajee A (2006) Dynamics of initial subgingival colonization of 'pristine' peri-implant pockets. Clin Oral Implants Res 17(1):25–37
33. Danser MM, van Winkelhoff AJ, de Graaff J, Loos BG, van der Velden U (1994) Short-term effect of full-mouth extraction on periodontal pathogens colonizing the oral mucous membranes. J Clin Periodontol 21(7):484–489
34. Lau L, Sanz M, Herrera D, Morillo JM, Martin C, Silva A (2004) Quantitative real-time polymerase chain reaction versus culture: a comparison between two methods for the detection and quantification of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythensis* in subgingival plaque samples. J Clin Periodontol 31(12):1061–1069
35. Keller W, Bragger U, Mombelli A (1998) Peri-implant microflora of implants with cemented and screw retained suprastructures. Clin Oral Implants Res 9(4):209–217
36. Lazarevic V, Whiteson K, Hernandez D, Francois P, Schrenzel J (2010) Study of inter- and intra-individual variations in the salivary microbiota. BMC Genomics 11:523
37. Elter C, Heuer W, Demling A, Hannig M, Heidenblut T, Bach FW, Stiesch-Scholz M (2008) Supra- and subgingival biofilm

formation on implant abutments with different surface characteristics. Int J Oral Maxillofac Implants 23(2):327–334
38. Burgers R, Gerlach T, Hahnel S, Schwarz F, Handel G, Gosau M (2010) In vivo and in vitro biofilm formation on two different titanium implant surfaces. Clin Oral Implants Res 21 (2):156–164
39. Preza D, Olsen I, Willumsen T, Grinde B, Paster BJ (2009) Diversity and site-specificity of the oral microflora in the elderly. Eur J Clin Microbiol Infect Dis 28(9):1033–1040
40. Lindhe J, Berglundh T, Ericsson I, Liljenberg B, Marinello C (1992) Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. Clin Oral Implants Res 3(1):9–16