Different implants have different biofilm communities—lessons for implant optimization
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Introduction

Micro-organisms use to live in communities forming self-structured aggregates. The aggregates, called biofilms, form at all kinds of interfaces, e.g. also on implants. Biofilm formation on implants is usually recognized as infection of the implants. Biofilm infections are difficult to treat due to their antibiotic resistances and novel approaches for their control are urgently needed. For finding new ways of biofilm control a deeper understanding of these biofilms and the risk factors for their formation is required. One open question was whether biofilm formation on implant always leads to infections. Furthermore, the species composition of the biofilm communities and their diversity between patients remained to be determined. To achieve this we assessed bacterial biofilm communities of rhythm management devices from asymptomatic patients using fingerprinting techniques based on the 16S ribosomal RNA (rRNA) genes and compared them with those found on symptomatic ones. These results were then compared with those from dental implants.

Methods

Explanted rhythmic management devices from patients not showing any sign of infections were examined. The reason for their explantation was malfunctioning of the devices or exhaustion of the batteries. The median interval between the implantation of the devices and their removal was 64 months. This drastically reduces the chance that any bacteria detected on the implants had been introduced by the first surgery. These asymptomatic devices were compared with devices from patients showing clear clinical signs of infections. Biofilm communities from asymptomatic and symptomatic dental implants and healthy teeth were taken as swaps with paper tips. All samples were immediately frozen at -80°C until DNA extraction.

The diversity of the biofilm communities were determined based on an amplicon of the 16S rRNA genes. Single strand conformation polymorphism (SSCP) analyses of these amplicons showed the diversity of bacterial communities while the sequence analyses of the main bands revealed the species compositions. Both SSCP gels and sequences were analysed using multivariate statistical methods.

**Figure 1**: Occurrence of biofilm bacteria on rhythmic management devices in relation to implant infections.
Results

From 108 rhythmic management devices in asymptomatic patients 47% had bacterial DNA and remained asymptomatic. The identified bacterial taxa were atypical for clinical device infections corroborating the clinical findings. No correlations were found between known risk factors for device infections and the bacteria detected [1]. The results were compared with rhythm management devices explanted due to acute infections revealing very different biofilm communities between these two types. Symptomatic biofilms had a much higher bacterial diversity and were dominated by Staphylococcus spp. [2]. The presence of many biofilm species correlated with the clinical symptoms of infections. While Staphylococcus epidermidis was exclusively found in symptomatic biofilms, Lactobacillus delbrueckii could only be detected in asymptomatic biofilms (Figure 1). Such a finding points to biofilm communities specific for asymptomatic implants which are replaced by different communities when the implants become infected. No conclusion could be drawn on the role of asymptomatic biofilms and whether they act protective to implant infections, neutral or supportive for the invasion of pathogens on implants. The fact that in more than 200 analysed biofilm communities from rhythmic management devices no transition from asymptomatic to symptomatic biofilm communities has been observed points to a development of pathogenic biofilm communities independent from the presence of asymptomatic ones.

Figure 2 Diversity of Streptococcus species observed in peri-implantitis patients. Sequences obtained from infected implants, OTU (=operational taxonomic unit) 1-9, are shown in a phylogenetic tree with their nearest Streptococcus species. Tannerella forsythia served here as outgroup. The bar shows 5% sequence similarity.
The formation of biofilms on rhythmic management devices occurs in a usually sterile environment. However, what is the course of biofilm development in an environment which is heavily populated by a broad range of bacteria species, e.g. in the oral cavity? Here, the implant will bridge the region of sterile tissue or bone to the unsterile oral cavity where the gingiva has a barrier function. To address these questions we compared the results from the pacemakers with asymptomatic and symptomatic biofilms from dental implants. Infection of dental implants leads to peri-implantitis which is a serious infection with incidence rates of up to 30%. Such an infection may cause peri-implant bone loss and implant failure. As a consequence, several approaches have been taken to reduce biofilm formation on dental implants. As for other implants assessment of biofilm diversity and variations between patients is required for the optimal design of anti-biofilm strategies. Our analyses revealed that biofilms from asymptomatic dental implants showed a large diversity of bacterial species comprising many phyla of bacteria. The species composition of these biofilms was also rather diverse between patients where each patient has his own characteristic biofilm community. The subgingival communities could be discerned by their species composition from those of supragingival and implant communities. However, the biofilm composition of healthy residual teeth showed not significant differences to the former three implying closely related biofilms both on residual teeth and on implants [3].

As for the rhythmic management devices it was important to determine the differences between asymptomatic and symptomatic biofilms on dental implants. From 10 patients with infected implants leading to peri-implantitis, the sulcus fluid of the infected implants and if available those of healthy implants and residual teeth were sampled. From the biofilm communities more than 60 different species could be identified revealing a large diversity of bacteria involved in peri-implantitis. As has been observed for the rhythmic management devices the diversity of bacteria on infected dental implants was much higher than on healthy ones. In Figure 2 the diversity of Streptococcus species detected both in asymptomatic and symptomatic biofilms on dental implants is shown. Only species related to S. mitis, S. intermedius and S. anginosus could be detected in biofilms on healthy implants while OTU 3 and OTUs 7-9 were specific for pathogenic biofilm communities. Interestingly, the biofilm communities from infected and healthy implants from the same patient were more similar than those of infected implants of different patients. Obviously, pathogenic biofilm communities spread in the oral cavity but cause only infections on some implants. Contrary to the rhythmic management devices no clear pathogen was found common to most of the infections.

Figure 3: Bacteria species of the phylum Bacteroidetes found in peri-implantitis samples. The long branches to known species point to novel species, maybe even novel genera in these complex pathogenic biofilm communities.
Conclusion

Currently, most infected implants are only examined for some pathogenic bacteria ignoring the majority of micro-organisms present on the implant. Such an approach proved to be very successful for finding the most effective antibiotics for the treatment. However, the finding of complex bacterial communities both on asymptomatic and symptomatic implants as demonstrated here leads to the question for the function of these communities, especially their role in the infection process. One promising way for answering this question is a correlation of clinical data of the patients with the community composition of the infected implant. This requires well defined large cohorts of patients, now available in some huge international studies. The hypothesis here is that an understanding of the interactions between non-pathogenic and pathogenic bacteria in biofilms on implants will open new ways of control of biofilm infections. Furthermore, a large number of hardly known bacteria can be detected in many of these biofilms, e.g. species of the bacterial phylum Bacteroidetes. Many of these bacteria present novel species or even belong to novel genera (Figure 3). Optimized isolation procedures and characterization of these new isolates will shed light on their function in pathogenic biofilm communities and their impact on the infections. The results from the rhythmic management devices also suggest that it is not essential to suppress any biofilm formation but only bacterial species characteristic for pathogenic biofilms. Here, surface coatings optimized for the suppression of the attachment of these pathogens or antibiotics specifically targeting these bacteria are probably sufficient for a significant suppression of biofilm infections at this type of implants. Also compounds which can dissolve the biofilm aggregates restoring the susceptibility of the pathogen for antibiotics may offer promising solutions [4]. Such solutions, however, will probably not work for implant infections where no pathogens common for all infections can be identified, e.g. for dental implants. The finding of very diverse microbial communities in peri-implantitis fits to a breakdown of the barrier function of the gingiva causing the disease. In this case non-selective, antimicrobial coatings of the implants are more promising solutions than the selective suppression of some pathogens. The detection of both implants with characteristic pathogens, here Staphylococcus species, and implants with no specific pathogens is challenging and suggests individual solutions. The results demand implants with antimicrobial characteristics dependent on the site of implantation. Furthermore, keeping in mind that patients have their individual biofilm communities all this will lead to „personalized“ implants which are optimized for the need of each patient. This probably will not lead to tailor-made implants but to different classes of implants where the antimicrobial coating meets the requirement of both the patient and the site of implantation. To achieve this goal we have still much to learn about pathogenic biofilm communities and the roles of their individual members.

References


