Case Control Study to Evaluate the Role of Candida, Staphylococcus and Enterococccus Species in Peri-implant Infections in Irradiated Patients after Tumour Surgery and Non-irradiated Patients

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Abstract

Background: The purpose of the study was to evaluate the micro-flora of healthy (P-) and infected implant sites (P+) in patients with a former radiation therapy after tumour surgery (R+) in comparison with patients without history of irradiation in the head and neck region (R-), focusing on *Candida, Staphylococcus and Enterococcus* species.

Methods: Patients with healthy implant sites (n=14; group I: P-; R-); with clinical signs of peri-implant infections (n=13; group II: P+; R-); with healthy implant sites after irradiation (n=7; group III: P-; R+) and with clinical signs of peri-implant infections after irradiation (n= 6; group IV: P+; R+) took part in this case control study. An oral assessment was performed for each patient, including: plaque index, sulcular bleeding-index, pocket probing depth, and bone loss. Samples out of the peri-implant sulcus have been used to identify periodontal pathogens, *Candida*, *Staphylococcus* and *Enterococcus* species, and testing their resistance to antibiotics/antimycotics.

Results: The most periodontal pathogens, especially *Tannerella forsythia* and *Fusobacterium nucleatum/periodonticum*, were found in patients of group II (P+; R-). *Candida*, *Staphylococcus* and *Enterococcus* species were detected in all patients groups. Multi-resistant *Candida* and *Enterococcus* species were found independently of the group of patients, however no multi-resistant *Staphylococci* could be seen.

Conclusions: Peri-implant infections occurred in patients with a former radiation therapy, but the number and composition of periodontal pathogens are lower compared with patients without irradiation. Independently of clinical signs of peri-implant infections *Candida*, *Staphylococcus* and *Enterococcus* species were present in all patients groups in the peri-implant sulcus, but multi-resistance was only detected in low numbers. It puts the role of these bacteria and yeast in question, since they were found in all patients groups.

Key Words: Peri-implantitis, Healthy implant sites, Radiation therapy, Candida, Staphylococcus and Enterococcus species, Multi-resistance

Introduction

Peri-implantitis is defined as a condition of inflamed peri-implant soft tissue associated with a loss of supporting bone around an implant in function [1,2]. In patients the prevalence of peri-implantitis varies between 28% and 56% and in implant sites between 12% and 43% [3-5]. In patients with radiation therapy of the head and neck region in their past medical history similar numbers of peri-implant infections (12%) have been described in their medical history like in patients without radiation therapy [6]. A cause and effect relationship between biofilm formation on implants and peri-implant mucositis, the reversible infection of the implant surrounding soft tissue, could be demonstrated by Pontoriero *et al.* [7] and Zitzmann *et al.* [8]. The peri-implant mucositis can lead to peri-implant infections with irreversible loss of bone. The micro-flora in infected implant sites is dominated by Gram-negative obligate anaerobic rods, fusiform bacteria and spirochetes, like species of *Porphyromonas*, *Tannerella* or *Treponema* [9,10]. Thereby, healthy implant sites normally are populated by high proportions of Gram-positive coccoid bacteria [11-15]. However, several studies showed that occasionally *Candida* species, *Staphylococcus* species and *Enterococcus* species are part of the peri-implant flora in infected peri-implant sites [10,16-21]. It is not well examined, if the micro-flora of infected and healthy implant sites differs in patients with and without former radiation therapy. However, a history of radiation therapy was detected as a factor influencing the development of clinical signs of peri-implant mucositis. But irradiation in the past failed to be a relevant factor for the detection of periodontal pathogens at the implant site [22]. *Candida, Staphylococcus and Enterococcus* colonization can be found in the oral cavity of patients after irradiation in higher amounts than in patients without radiotherapy [23]. If these micro-organisms also occur more frequently in the peri-implant sulcus in peri-implant infections in patients after radiation therapy is not known. Independent of a former radiation therapy, in patients with peri-implantitis the role of S*taphylococcus* species*, Enterococcus* species and *Candida* species remains also still unknown. No data on the susceptibility of these bacteria and yeasts of the peri-implant sulcus to antibiotics, respectively antimycotics, are available.

The purpose of the present study was to evaluate the micro-flora of patients with healthy and peri-implant infected implant sites with and without a former radiation therapy in the head and neck region after tumour surgery. The main focus has been the detection of *Candida*, *Staphylococcus* and *Enterococcus* species in the peri-implant sulcus to clarify the role of these pathogens. In addition these species were tested for their

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susceptibility to different antibiotic, respectively antifungal substances.

Method

For this study, 40 patients enrolled in the routine implant recall were screened in the Department of Oral and Maxillofacial Surgery of the University Medical Center of the Johannes Gutenberg University of Mainz, Germany. The study protocol was approved by the local ethics committee (873.431.06 (5529) and written informed consent was obtained from each patient. The same physician carried out all investigations and diagnostic procedures. Inclusion criteria for this case control study: removable restoration in situ for ≥6 months, being edentulous and having implants in the mandible. For patients after radiation therapy the volume had to include the mandible, the total submandibular region and the salivary glands, and the cranial border of the radiation field had to be above the chin-mastoid plane [24]. The radiation dosage varied between 50-70 Gy per patient in fractions of 2 Gy per day after surgical tumour therapy. All tumour patients underwent surgical and conservative teeth rehabilitation in the planning phase of radiotherapy routinely. Exclusion criteria: usage of antibacterial mouthwash 24 hours before examination, an antibiotic therapy three weeks prior to examination, a steroid treatment or chemotherapy in the last three months or being an immuno-compromised patient (with HIV infection or leukaemia).

An oral assessment was performed for each patient, including the determination of the following clinical parameters of the implant site:

- modified plaque index (PI): plaque absent or present [25]
- modified sulcular bleeding-index (BOP): bleeding on probing absent or present [26]
- pocket probing depth (PD): measured per implant with a periodontal probe. (plast-o-probe periodontal probe, Dentsply de Trey, Germany) at six sites around the implant. The highest value was chosen per implant.
- bone level: measured on panoramic radiographs as the distance between implant shoulder and first bone-to-implant contact. For each implant, the highest, most unfavourable value of the mesial and distal measurement was recorded [27].

Patients with one implant site tested positive for plaque, BOP, PD ≥5 mm, and bone loss were termed as having an "inflammation at the implant site"[28]. Patients have been divided into four groups.

- group I: no clinical signs of a peri-implant infection (P-); no radiotherapy in the past (R-)
- group II: clinical signs of a peri-implant infection (P+); no radiotherapy in the past (R-)
- group III: no clinical signs of a peri-implant infection (P-); radiotherapy in the past (R+)
- group IV: clinical signs of a peri-implant infection (P+); radiotherapy in the past (R+)

For each patient the implant with the deepest pocket was chosen for the microbiological analysis. Bacterial samples were obtained after removing the supra-gingival plaque with a light curette for implants (Straumann, Germany) and drying the surface of the implant. According to the manufacturer, a sterile paper point, included in the Hain kit, was inserted into the peri-implant pocket, where it was left in place for 10s. The paper points were placed into sterile tubes. One sample was sent to a laboratory for DNA analysis (micro-IDent®, HAIN LIFESCIENCE, Nehren, Germany). Eleven periodontal pathogens were included in the DNA analysis (*Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, *Treponema denticola*, *Peptostreptococcus micros*, *Fusobacterium nucleatum/periodonticum*, *Campylobacter rectus*, *Eubacterium nodatum*, *Eikenella corrodens*, *Capnocytophaga spec*.). A second sample was used for the microbiological cultural analysis of *Candida*, *Staphylococcus* and *Enterococcus* species being present at the implant site. Therefore the paper points were placed into sterile tubes containing sterile saline and processed immediately. Aliquots of 0.1 ml were streaked on agar plates, prepared from Sabouraud agar (Oxoid, Wesel, Germany) for detecting *Candida* species. Mannitol Salt Agar (MSA; Merck, Darmstadt, Germany) was used for detecting *Staphylococcus* species and Chromocult® enterococci agar (Merck, Darmstadt, Germany) for detecting *Enterococcus* species. The plates have been incubated in an aerob atmosphere at 37°C. After 24-48 hours, the plates were taken out and inspected. Colonies growing on the different selective breeding grounds have been taken from the plates for purification and further characterization on Brain Heart Infusion (BHI) broth (Becton Dickinson, Heidelberg, Germany).

After purification of the samples DNA was extracted using QIA quick PCR Purification Kit (Qiagen), following the manufacturer's instructions. Quantification of bacterial DNA were determined with a Qubit™ Fluorometer und Quant-iT™ dsDNA BR Assay Kits (Invitrogen™, Paisley, USA) at 510/527 nm.

In order to generate standard curves for the PCR and positive controls reference micro-organisms were obtained from the Leibniz Institute German Collection of Microorganisms and Cell Cultures (DSMZ): *Enterococcus faecalis* (Nr. 20478), *Enterococcus faecium* (Nr. 20477), *Staphylococcus aureus* (Nr. 20231), *Staphylococcus epidermidis* (Nr. 20044), *Candida albicans* (Nr. 1386) and *Candida glabrata* (Nr. 6425). DNA was extracted from the reference stock samples and clinical samples with the QIA ampDNA MiniKit (Qiagen, Valencia, USA) according to the manufacturer's instructions.

Fragments of all tested microbial species were generated by PCR in a Thermocycler (Eppendorf, Hamburg, Germany). The PCR cycle parameters were as follows: thermal activation for 10 minutes at 95°C and 35 cycles of PCR (denaturation for 45 seconds at 94°C, annealing for 45 seconds at 55–65°C, and extension for 60 seconds at 72°C). To verify the specificity of the PCR reactions, PCR products were electrophoresed alongside the 50-bp DNA Molecular Weight Marker XIII (Roche Diagnostics, Mannheim, Germany) through a 2% (w/v) agarose gel (Invitrogen). The gels were stained with SYBR green (Roche), and images were captured using a Kodak EDAS 120 Image System (Eastman Kodak Sa`rl, Gene`ve, Switzerland). The PCR products were purified with

the QIA quick PCR Purification Kit (Qiagen) according to the manufacturer's instructions, and the DNA concentrations were determined using a NanoVue system (GE Healthcare). The copy number was calculated and serial 10- fold dilutions were made in the range of 1×10^1 to 1×10^7 copies [29].

After identification the isolates as well as the test bacteria were mixed with top agar and poured onto agar plates to determine possible resistance of the strains against specific antibiotics and antimycotics. The susceptibility of the cultures was tested against amoxicillin, amoxicillin/clavulanic acid, ampicillin, ampicillin + sulbactam (2:1), penicillin, azithromycin, linezolid, and minocyclin in the concentration of 0.016-256 µg/ ml and moxifloxacin in the concentration of 0.002-32 µg/ml using the Etest®® (AB BIODISK, Dalvägen, Solnam Sweden). The yeasts were tested against amphotericin B, ketoconazol and voriconazol in a concentration of 0.016-32 µg/ml and fluconazole in a concentration of 0.016-256 µg/ml. The minimum inhibitory concentration (MIC) was measured after 24-48 hours of incubation according to the manufacturers' instructions. The MIC values have been divided into susceptible, intermediate susceptible or resistant using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines as reference.

Statistical Analysis

Data collection, data management and data analysis were performed with the statistical software package SPSS® Version 21. For the qualitative variables, absolute and relative frequencies were calculated. Values were given as mean and standard deviation. The Kruskal Wallis test and Mann-Whitney U test was used to test for possible statistical significance. A p value <0.05 was considered as statistically significant.

Results

Patient collective

Out of the study sample of 40 patients, 14 patients (35%) belonged to group I (P-; R-), and 13 patients (33%) to group II (P+; R-). Seven patients (18%) have been assigned to group III (P-; R) and six patients (15%) to group IV (P+; R+). Looking at the age and the gender of the patients, there was no difference of the patients collective comparing the four groups. The average length after the prosthetic reconstruction differed between the groups with and without radiation therapy. In patients with a former radiation therapy (group III, and group IV) the restoring time was much shorter than in the other groups (p = 0.002). Most smokers belonged to group II without a statistical significance *(Table 1)*.

Description of peri-implant pathogens present at the implant site in the different patient groups

Periodontal pathogens were found in 7/14 (50%) implant sites of group I (P-; R-), and in 12/13 (92%) implant sites of group II (P+; R-). Whereas in patients with a former radiation therapy in the past in 2/7 (29%) implant sites (group III: P-; R+), respectively in 4/6 (67%) implant sites (group IV: P+; R+) periodontal pathogens could be detected *(Table 2)*.

Table 1. *Patients collective.*

	Group P^-R^- (n = 14)	**Group P^+R^- (n = 13)**	**Group P^-R^+(n = 7)**	**Group P^+R^+(n = 6)**
Age	68 ± 4.61; (63-81)	68 ± 7.25; (56-79)	65 ± 13.50 (54-75)	68 ± 12.29 (53-81)
Gender (male/female)	6/8	4/9	6/1	3/3
Average length after prosthetic reconstruction (years)	14 [11-15]	14 [11-16]	2 [2-10]	4 [2-9]
Radiation dosage (Gy)	0	0	60 [60-60]	60 [60-60]
Smoking (number of patients)	3	7	2	1

Table 2*. Clinical signs of peri-implant inflammation at the implant site, where the microbiologic samples were obtained.*

	Group P^-R^- (n = 14)	**Group P^+R^-(n = 13)**	**Group P^-R^+(n = 7)**	**Group P^+R^+(n = 6)**
PI positive	11 (79%)	13(100%)	3 (43%)	6 (100%)
BOP positive	3 (21%)	13 (100%)	2 (29%)	6 (100%)
PD (mm, mean, SD)	2.6 (± 0.9)	5.5 (± 1.5)	2.6 (± 1.1)	5.2 (± 0.4)
BL (mm, mean, SD)	0.94 (± 1.37)	2.31 (± 1.57)	0.55 (± 0.60)	1.18 (± 1.04)

Table 3. Number of patients with positive micro-organisms on the implant sites and range of the concentration of the micro-organisms.

	Group P^-R^-(n = 14)	**Group P^+R^-(n = 13)**	**Group P^-R^+(n = 7)**	**Group P^+R^+(n = 6)**
Aggregatibacter actinomycetemcomitans	0	1 (= 10^4)	0	0
Porphyromonas gingivalis.	2 (< 10^5 - < 10^6)	4 (= 10^4 - < 10^6)	0	1 (= 10^4)
Tannerella forsythia	0	4 (= 10^4 - < 10^6)	0	0
Treponema denticola	0	3 (= 10^4 - < 10^6)	0	0
Prevotella intermedia	0	3(= 10^4 - > 10^7)	0	0
Micromonas micros	3 (< 10^5)	7 (= 10^4 - < 10^6)	0	1(= 10^4)
Fusobacterium nucleatum	6 (= 10^4 - < 10^6)	12 (< 10^5 - > 10^7)	2 (< 10^5 - < 10^6)	4 (= 10^4-< 10^5)
Campylobacter rectus	1 (< 10^6)	2 (< 10^6)	0	0
Eubacterium nodatum	0	1 (= 10^4)	0	0
Eikenella corrodens	5 (= 10^4 - < 10^6)	6 (= 10^4 - < 10^6)	0	2 (= 10^4)
Capnocythophaga spp.	4 (= 10^4 - > 10^7)	4 (= 10^4 - < 10^6)	1 (< 10^6)	0

Table 4. *Numbers of bacteria and yeast with a positive growth on the selective culture medium.*

	Group P^-R^-(n = 14)	**Group P^+R^-(n = 13)**	**Group P^-R^+(n = 7)**	**Group P^+R^+(n = 6)**
Candida species	2 (14%)	3 (23%)	1 (14%)	3 (50%)
Staphylococcus species	2 (14%)	1 (8%)	1 (14%)	1 (17%)
Enterococcus species	11 (71%)	10 (69%)	6 (71%)	5 (67%)

Table 5. *Specific primer and samples and optimized temperatu e conditions for PCR.*

PCR assay (amplicon size, annealing temp)	Oligonucleotide Sequence (5'-3')	Reference
Staphylococcus aureus (279 bp, 64°C)	Forw: 5'-GATTGATGGTGATACGGTT-3' Rev: 5'-AGCCAAGCCTTGACGAACTAAAG-3'	PMID: 1629319 [35]
Staphylococcus epidermidis (125 bp, 60°C).	Forw:5'-ATCAAAAAGTTGGCGAACCTTTTCA-'3 Rev: 5'-CAAAAGAGCGTGGAGAAAAGTA-'3	PMID: 1629319 [35]
Candida glabrata (127 bp, 60°C)	Forw: 5'-AAGAAGGCTGCCTGTTGTAATG'-3 Rev:5'-AACCAAGTATGCAGGGTCTGTT-'3	PMID: 11526177 [36]
Candida albicans (273 bp, 58°C)	Forw:5'-TTTATCAACTTGTCACACCAGA-'3 Rev:5'-ATCCCGCCTTACCACTACCG-'3	PMID:15713607 [37]
Enterococcus faecalis (357 bp, 60°C)	Forw: 5'-AACCTACCCATCAGAGGG-'3 Rev: 5'-GACGTTCAGTTACTAACG-'3	PMID: 15184159 [38]
Enterococcus faecium (75 bp, 62°C)	Forw: 5'-TTCTTTGCTTTATCCGATGT-'3 Rev: 5'-CGGTTTTCTGCTTTTGTAAT-'3	PMID: 14742209 [39]
Enterococcus spp. (144 bp, 68°C)	Forw:5'-CCCTTATTGTTAGTTGCCATCATT-'3 Rev: 5'-ACTCGTTGTACTTCCCATTGT-'3	PMID: 15546407 [40]
Universal U16S: (170 bp, 60°C)	Forw: 5'-TTAAACTCAAAGGAATTGACGG-'3 Rev: 5'-CTCACGRCACGAGCTGACGAC'-3	PMID: 15848151 [41]

Forw = sense primer; Rev = anti-sense primer; PMID = PupMed identifier

The highest amount of periodontal pathogens could be found in patients with peri-implantitis without radiation therapy (P+; R-; p = 0.007; *Table 3*). The periodontal pathogens *Tannerella forsythia* (*T.f.*) (p=0.029) and *Fusobacterium nucleatum/periodonticum* (*F.n.*) (p=0.018) dominated.

Description of Candida, Staphylococcus and Enterococcus species

Candida species were found in nine samples (*Candida albicans* n=8; *Candida glabrata* n=1). *Staphylococcus* species were found in five samples *(Staphylococcus epidermidis* n=2; *Staphylococcus aureus* n=3). *Enterococcus* species were found in 29 samples (*Enterococcus faecium* n=7; *Enterococcus faecalis* n=12; *Enterococcus species* n=10) taken from the peri-implant sulci. *Candida, Staphylococcus* and *Enterococcus* species were detected in all four of the patients groups, in 8% -71% of the patients (Table 4).

Susceptibility of the Candida, Staphylococcus and Enterococcus species

Eight *Candida* species 8/9 (89%) were resistant against FL, VO and KE (256 µg/ml). Out of these eight strains, one strain was susceptible to AP (*Candida albicans*), four strains showed intermediate susceptibility and three have been resistant to AP (two *Candida ablicans*; one *Candida glabrata*). Therefore, these three strains showed multi-resistance against the tested antifungal agents. The test strain *Candida albicans* was susceptible against the tested antimycotics and *Candida glabrata* was susceptible against AP, VO and intermediate susceptible against FL, and KE.

Two *Staphylococcus epidermidis* and one *Staphylococcus aureus* were resistant (256 µg/ml) to azithromycin. The test strains S*taphylococcus aureus* and *Staphylococcus epidermidis* were susceptible to the nine tested antibiotics.

4/32 (13%) *Enterococcus* isolates showed a resistance against the tested antibiotics (two *Enterococcus faecalis,* two *Enterococcus* species). They were identified and treated as multi-resistance strains. The two test strains (*Enterococcus faecalis*, *Enterococcus faecium*) have been susceptible to the tested antibiotics.

No difference of susceptibility of the tested *Candida, Staphylococcus* and *Enterococcus* species against the antibiotics, respectively antimycotics was present in the four groups of patients *(Table 5).*

Discussion

The peri-implant sulcus of patients with a peri-implant infection harbored more periodontal pathogens with higher total numbers of bacteria in contrast to patients with healthy implant sites. Thereby in patients with a past radiation therapy periodontal pathogens were detected in lower levels in the peri-implant sulcus, independent of clinical signs of peri-implant infections on the implant site. *Candida*, *Staphylococcus* and *Enterococcus* species were found in the peri-implant sulcus in all four of the patients groups. In low numbers multi-resistant *Candida* and *Enterococcus* species occurred in the peri-implant sulcus, independent of clinical signs of a peri-implant infection or irradiation.

The finding of high proportions of periodontal pathogens at the implant site in patients with peri-implantitis without irradiation is comparable to the study of da Silva *et al.* (2013) and others [9,11,12]. Da Silva *et al.* (2013) used PCR amplification of universal 16S rRNA to identify bacteria from healthy and infected implant sites out of the supragingival

plaque taken with a curette [30]. They identified the bacteria to the genus level and found similar high bacterial loads of *Tannerella forsythia* and *Fusobacterium nucleatum* in infected implant sites.

One explanation for the reduced detection of periodontal pathogens in the peri-implant sulcus of patients after radiation therapy could be the difference of the surgical and conservative teeth rehabilitation in the planning phase of the radiotherapy. This might influence the comparison of the peri-implant micro-organisms. However, the difference in the frequency of periodontal pathogens in comparison of peri-implant infected patients with and without radiation therapy might imply that signs of peri-implant infections occur after radiation therapy earlier and do not depend fundamentally on periodontal pathogens. A radiation dose over 50 Gy in the head and neck region was described as a negative co-factor for the development of peri-implant mucositis, not being linked to periodontal pathogens in the peri-implant sulcus [22]. Kwon *et al.* (2010) found *Candida* species in irradiated and non-irradiated; as well as in healthy (36%) and infected (64%) implant sites in tumor patients [31]. They identified *Candida glabrata* and *Candida albicans* and in one patient *Candida tropicalis* in the peri-implant sulcus similar to the results in the present study. Patients with a former radiation therapy showed a higher quantity of oral *Candida* colonization (83%) than patients without a radiation therapy in the past (58%) [32,33]. But, it is unclear if the amount of oral *Candida* colonization affects the micro-flora of the peri-implant sulcus. In contrast to the present study, Leonhardt et al. (1999) detected *Candida* species only sporadically in the supragingival plaque of peri-implant lesions, preferentially in partial edentulous patients and none in healthy subjects [18]. Alcoforado *et al.* (1991) and Listgarten *et al.* (1999) described the finding of *Candida albicans* in the peri-implant sulcus of failing implants [16,17]. It is unclear if the numbers of *Candida* species increases in the peri-implant sulcus after antibiotic therapy of peri-implant infections. But it is to be expected that a change of the micro-flora of the sulcus, from which *Candida* species benefits.

The very low number of *Staphylococcus* strains in the peri-implant sulcus in infected implant sites is comparable with other studies [16-18]. Examining healthy and infected implant sites, Persson et al. (2013) were able to find significantly higher levels of *Staphylococcus aureus* among other bacteria in implant sites with peri-implantitis [20]. The quantity of *Staphylococcus* species in the peri-implant sulcus was not evaluated in the present study. Therefore it is not possible to compare the present data with the study of Persson et al. (2013). The detection of *Staphylococcus* species in healthy peri-implant sulcus was not confirmed in other studies. However, the bacterial load of *Staphylococcus* species in the peri-implant sulcus might be more important for the development of a peri-implant infection than the simple presence of these strains.

The appearance of *Enterococcus* species in the peri-implant sulcus has been described already in patients with peri-implant infections [17,18,34]. The high percentage of *Enterococcus* strains in the healthy peri-implant sulcus was not confirmed in other studies. The role of these *Enterococcus* strains in the development of peri-implant infections is still unclear.

A limitation of the study might be the small size of samples in each of the different groups of patients, which can lead to a reduction of the explanatory power of the study. The small sample sizes can be explained by the decision to include only edentulous patients with implants in the mandible. The reason for choosing only edentulous patients was that the type of dentition might be a risk factor for peri-implant infections. Increased proportion of periodontal pathogens can be found in dentate patients compared with edentulous patients, because the periodontal pockets serve as reservoirs for these periodontal pathogens [22]. To reduce the number of influencing factors and to make the microbiological results comparable between the groups, only edentulous patients were included. Most edentulous patients with a former radiation therapy and hyposalivation need implants in their lower jaw to secure the hold of the prosthesis. Therefore more edentulous patients with implants in the lower jaw were available for an examination of the peri-implant micro-flora. Not to minimize the sample sizes of the different groups again, patients with implants in the maxilla were not included.

Growing concerns are based on the detection of multi-resistant strains of the peri-implant sulcus. But the amount of resistance was very low looking at the *Staphylococcus* and *Enterococcus* species. The clinical implication of the resistant *Candida* species in the peri-implant sulcus is still unclear. It could be seen that the antimycotics based on azole are less effective against *Candida* strains than polyene antimycotics. To which extend a possible gene transfer between such yeasts, bacteria and true pathogens in the peri-implant sulcus may lead to resistant strains has to be focused on in further studies.

Conclusion

In patients with a former radiation therapy clinical signs of peri-implant infections appear often with a lower number of periodontal pathogens in the peri-implant sulcus than in patients without radiation therapy. *Candida*, *Staphylococcus* and *Enterococcus* species occur in healthy and infected implant sites in patients with and without irradiation. The detection of these bacteria and yeasts in all patients groups questions their role in the development of peri-implant infections. In further studies it should be focused if the quantity of *Candida* species might play a role in the development on peri-implant infections in patients after radiation therapy.

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