GENERAL DISCUSSION

At the start of this PhD project in 2008, the knowledge on peri-implant infections was rather limited. A PubMed search on the term 'peri-implantitis' yielded approximately 200 studies. By the beginning of 2015 this number had increased to over 1000 studies, indicating that the dental field is becoming more and more aware of the phenomenon of 'peri-implantitis'. Despite this increased awareness the existing knowledge on peri-implant infections is still not overwhelming, especially in comparison to the existing knowledge on periodontal infections (40,000 PubMed hits on the term 'periodontitis'). A famous periodontist once said: 'In periodontology we know a lot more than we practice'. Later a famous microbiologist stated: 'In implantology we practice a lot more than we know'. The aim of this thesis was to add to the knowledge on peri-implant infections, with emphasis on the epidemiology, microbiology and treatment.

Epidemiology

In chapter 2, it was shown that peri-implantitis is not very likely to occur within the first 5 years of implant functioning. However, after a functional period of ≥10 years peri-implantitis is observed in 6 to 17% of the implants. Unfortunately, only data on the prevalence of peri-implantitis in partially edentulous subjects were available, but recently it has been shown that both peri-implant mucositis and peri-implantitis also occur frequently in fully edentulous patients, at rates comparable to partially edentulous subjects (Meijer et al. 2014). It seems reasonable to expect that the prevalence of peri-implant infections will further increase with increasing implant function time. This, combined with the notion that the number of patients with dental implants is continuously increasing, implies that we will be confronted with peri-implant infections more and more.

When comparing prevalence numbers of peri-implant infections it is important to consider 1) the threshold levels used for defining peri-implant mucositis and peri-implantitis, 2) the difference between patient level and implant level reported outcomes, 3) the difference between incidence and prevalence and 4) the characteristics of the patients included, for example with regard to smoking habits and history of periodontitis. These factors, amongst others, significantly influence the reported outcomes (Roos-Jansåker et al. 2006, Roccuzzo et al. 2010, Simonis et al. 2010, Koldsland et al. 2010, Meijer et al. 2014).

In chapter 2 it was further shown that no differences exist in implant survival rate and mean probing pocket depth between fully edentulous and partially edentulous subjects. Data regarding peri-implant mucosal bleeding were inconsistent, probably due to the fact that studies directly comparing fully and partially edentulous subjects were not available. The only difference in clinical status that could be found between fully and partially edentulous subjects was the amount of plaque at the implants, which was consistently significantly higher in fully edentulous subjects. It was of interest to note that a higher plaque level did not translate into more disease.

Microbiology

Whether or not the composition (quality) of the plaque, in addition to the quantity of the plaque, would also differ between fully and partially edentulous implant patients, was investigated in chapter 3. It appeared that in healthy and peri-implant mucositis

conditions partially edentulous subjects harbor a potentially more pathogenic peri-implant microflora than fully edentulous subjects, which means that these subjects harbor higher proportions of bacteria that have been associated with periodontal and peri-implant disease (Mombelli et al. 1987, Zambon 1996, Leonhardt et al. 1999, Shibli et al. 2008). This is in line with the observation in chapter 4 that the oral microflora is significantly influenced by the presence or absence of teeth. Significant changes occur in the oral microflora as a result of full-mouth tooth extraction. These changes included reduction of *A. actinomycetemcomitans* and *P. gingivalis* frequently to levels below detection threshold. Apparently, these changes in the oral microflora after full-mouth tooth extraction persist to some extent (at least for a considerable amount of time) after implant installation (chapter 3). These observations strengthen the hypothesis, put forward in chapter 3, that the higher plaque levels observed in fully edentulous implant patients are counterbalanced by the reduced numbers of putative periodontal pathogens, resulting in implant survival rates that are comparable to partially edentulous implant patients.
Remarkably, the case-control study described in chapter 5 revealed very low detection frequencies of *A. actinomycetemcomitans*, both in health (1%) and disease (3%). Consistently, *A. actinomycetemcomitans* was only rarely detected in patients that were treated for peri-implantitis (chapters 6 and 7), possibly suggesting that the role of this periodontal pathogen in peri-implantitis is limited. The low prevalence of *A. actinomycetemcomitans* in peri-implantitis lesions may be the result of strict assessment and selection of patients applying for implant treatment. Patients with (a history of) aggressive periodontitis, which is the form of periodontitis that is most specifically associated with *A. actinomycetemcomitans* (Henderson et al. 2010), may frequently be excluded from implant treatment because of the increased risk for development of peri-implantitis compared to non-periodontitis patients or patients with (a history) of chronic periodontitis (Sgolastra et al. 2015). *A. actinomycetemcomitans* may also be relatively easily eliminated from the oral cavity prior to implant placement as a result of full-mouth tooth extraction (chapter 4) or as a result of successful periodontal treatment including combination therapy of amoxicillin and metronidazole (Van Winkelhoff et al. 1992, Winkel et al. 2001, Guerrero et al. 2014).

In chapter 5, significant associations between peri-implantitis and submucosal presence of *P. gingivalis, P. intermedia, T. forsythia and F. nucleatum* were noted. These observations are in line with most other studies using culturing or targeted molecular analyzing techniques, such as checkerboard DNA-DNA hybridization and polymerase chain reaction, for comparison of biofilms associated with peri-implant health and disease (Salcetti et al. 1997, Leonhardt et al. 1999, Hultin et al. 2002, Botero et al. 2005, Shibli et al. 2008, Máximo et al. 2009, Cortelli et al. 2013, Tamura et al. 2013, Persson & Renvert 2014). When interpreting the results of the above mentioned studies, one should keep in mind that these studies are all cross-sectional. Long-term longitudinal studies with large populations of implant patients with different initial microbial profiles, which would be necessary for establishment of true causative relationships between microbiological characteristics and peri-implant

disease onset/progression, have not been presented so far. Moreover, one may argue whether it is at all possible to establish causative relationships for a complex poly-microbial disease such as peri-implantitis, in which many different species of micro-organisms interact within a biofilm. Despite these limitations, it still seems rational to try to reduce the numbers of the known peri-implantitis associated pathogens in the oral cavity prior to implant installation and thereafter. Therefore, one risk-reducing measure could imply pre-operative microbiological screening in patients designated for implant treatment.
In general, infectious diseases develop when the 'minimum infectious dose' or the minimum amount of bacteria required to cause infection, is surpassed. This 'minimum infectious dose' may vary between individuals and probably depends on microbial factors, such as virulence and relative proportion of micro-organisms in a biofilm, and host-specific factors such as genetic composition, smoking and stress. Prevention of peri-implant infections should therefore be two-fold. First, the bacterial load challenging the peri-implant tissues should be kept below the 'minimum infectious dose'. This can be accomplished by the establishment of a prosthetic reconstruction that is in harmony with the surrounding tissues and is accessible for adequate plaque control, combined with proper oral hygiene measures by the patient. Second, the 'minimum infectious dose' should be maintained as high as possible, for example by smoking cessation and reduction of the numbers and proportions of virulent micro-organisms.

Up to now, research on peri-implant microbiology has mainly focused on periodontal pathogens, using both culturing and targeted molecular approaches. This focus on periodontal pathogens has been imposed by the perceived similarities between both diseases and the increased susceptibility of (treated) periodontitis patients to develop peri-implantitis (Sgolastra et al. 2015). Recently, the use of new techniques, employing an open-ended, global approach for the examination of microbial communities, has extended our knowledge on the peri-implantitis associated microbial community. Although the microbial associations that have previously been established based on conventional techniques generally still hold true when these new techniques are being applied (Kumar et al. 2012, Da Silva et al. 2014), it has been shown that micro-organisms usually not found in periodontal lesions can be encountered in peri-implant disease (Kumar et al. 2012, Da Silva et al. 2014). In addition, it has been suggested that the peri-implant and periodontal microbiome might not be as similar as previously thought (Kumar et al. 2012, Heuer et al. 2012, Dabdoub et al. 2013, Koyanagi et al. 2013). Peri-implant biofilms seem to demonstrate significantly lower diversity, with a lower number of species, than subgingival biofilms in both health and disease (Kumar et al. 2012, Heuer et al. 2012, Dabdoub et al. 2013), but contradicting results have been reported (Koyanagi et al. 2013). It has been suggested that peri-implantitis is a relatively simple infection, yet microbiologically heterogeneous among individuals (Kumar et al. 2012, Dabdoub et al. 2013).
The differences between the periodontal and peri-implant microbiome may be the result of differences in structure and material properties of the colonized surfaces that likely affect bacterial adhesion. In addition, the microbial differences may arise from the anatomical differences between the periodontal and peri-implant tissues and the

differences in inflammatory reactions of the tissues. Although the development of periodontitis and peri-implantitis lesions clinically seems to follow a similar sequence of events, the dynamics of the pathological processes may not be identical (Heitz-Mayfield & Lang 2010, Belibasakis et al. 2015).
Recently, it has been suggested that peri-implant bone loss may not primarily be the result of bacterial infection, but is primarily caused by a disturbance of the immune-mediated foreign body equilibrium (Albrektsson et al. 2014, Trindade et al. 2014). This foreign body equilibrium is supposedly achieved when integration of the dental implant in the bone occurs (osseointegration) and is characterized by a mild chronic inflammatory response, including regular presence of multinuclear giant cells at the implant interface, as a result of which the implant is shielded off from the rest of the organism by an enveloping bone tissue layer that gradually condenses (Albrektsson et al. 2014, Trindade et al. 2014). A disturbance of the foreign body equilibrium may lead to bone resorption and rupture of the mucosal coronal seal, which may consequently lead to a secondary bacterial infection of the peri-implant tissues (Albrektsson et al. 2014, Trindade et al. 2014). This hypothesis of an immune-modulated foreign body equilibrium, representing a delicate balance to avoid rejection of the dental implant (Albrektsson et al. 2014), combined with the structural differences between the peri-implant and periodontal tissues, such as limited vascularity, limited innervation, low fibroblast-to-collagen ratio and absence of a periodontal ligament, may explain why persistent biofilm accumulation seems to elicit a more pronounced inflammatory response in peri-implant tissues compared to periodontal tissues (Ericsson et al. 1992, Lindhe et al. 1992, Schou et al. 1993). In addition, peri-implantitis lesions may progress more quickly than periodontitis lesions because of the absence of a healthy connective tissue fiber compartment walling off the lesion from the alveolar bone, which may allow the infection to progress into the bone marrow in some instances (Heitz-Mayfield & Lang 2010). Taken together, it seems that the peri-implant tissues are less capable of dealing with a (microbial) challenge than the periodontal tissues, which may explain why peri-implantitis seems to be more difficult to treat successfully than periodontitis.

Treatment

The ultimate goal of treatment of peri-implant infection is complete resolution of the inflammatory lesion and re-osseointegration along the entire surface of the previously contaminated implant surface. However, re-osseointegration is difficult to achieve and is unpredictable (Renvert et al. 2009). Therefore, treatment of peri-implantitis usually focuses on resolution of inflammation and halt of progression of peri-implant bone loss. Implant surface decontamination represents an important step in peri-implantitis treatment. Ideally, the status of the implant surface is returned to the pre-implantation status, which is a sterile, non-contaminated, unaffected implant surface with a reestablished surface atomic composition and titanium oxide structure (Mouhyi et al. 2012). Additionally, for the preservation of the osteogenic potential of the peri-implant tissues, it is important that the treatment has no lethal effects on surrounding tissues (Mouhyi et al. 2012). To allow for optimal decontamination of the implant surface, it is recommended to perform access surgery (Lindhe et al. 2008). This facilitates proper

granulation tissue removal and fully exposes the screw-shaped and rough implant surface for decontamination. Non-surgical approaches for the treatment of peri-implantitis have shown to be unpredictable and, in most case, not sufficiently effective (Renvert et al. 2008, Esposito et al. 2012). However, it is recommended that surgical intervention is always preceded by non-surgical treatment, in order to optimize oral hygiene procedures, reduce the total bacterial peri-implant load and reduce signs of inflammation.

Due to the wide variation in materials and procedures that have been described for the decontamination of the implant surface and treatment of peri-implantitis, it has been difficult to discriminate between effective and ineffective (components of) interventions (Esposito et al. 2012, Heitz-Mayfield & Mombelli 2014). Therefore, it was suggested that it may be necessary to study simple interventions using a double-blind study design before gradually testing more complex treatments (Esposito et al. 2012). For that reason, in chapters 6 and 7 we explored the clinical and microbiological effects of implant decontamination using chlorhexidine (CHX), an antiseptic that is widely used in periodontology and has well-documented clinical efficacy. In our treatment studies no regenerative procedures were applied and systemic or local antibiotic therapy was not provided.

For evaluation of the direct microbiological effect of the decontamination procedure bacterial samples were taken from the implant surface using sterile brushes. This sampling method was developed for the specific purpose of the study with the intention to allow for sampling of the deep parts of the rough implant surface and between the threads, areas that would have been difficult to reach using conventional sampling methods such as paperpoints or curettes. It turned out that implant surface decontamination with 0.12% chlorhexidine (CHX) + 0.05% cetylpyridinium chloride (CPC) in addition to mechanical debridement of the implant surface during the resective surgical treatment procedure led to a greater immediate reduction of total anaerobic bacteria on the implant surface than the placebo solution (Chapter 6). Increasing the CHX concentration to 2% did not significantly increase the immediate antimicrobial effect of the debridement and decontamination procedure (Chapter 7). This could possibly indicate that the killing effect of chlorhexidine is time dependent rather than concentration dependent and that increasing the exposure time would be a better way to improve microbiological results compared to increasing the concentration. Overall, despite the immediate microbiological effect of CHX over placebo, the additional use of CHX did not enhance clinical treatment outcomes. No differences were observed in bleeding, suppuration, probing pocket depth and radiographic bone loss between the placebo group, the 0.12% CHX group and the 2% CHX group. One explanation for this observation could be the lack of cleaning capacity of CHX. Several *in vitro* studies have shown that CHX is effective in killing bacteria in biofilms grown on titanium surfaces (Chin et al. 2007, Gosau et al. 2010), but CHX seems only modestly effective in actually removing the biofilm (Ntrouka et al. 2011a). Another explanation could be that the success of peri-implantitis treatment is determined by factors other than the method of surface debridement and decontamination (Chapter 8). If treatment is aimed to halt further peri-implant bone loss, a clean ("pristine") implant surface might indeed not be a prerequisite for achieving a successful treat-

ment outcome. However, if re-osseointegration is the goal, a clean implant surface in addition to an unaffected implant topography seems mandatory (Mouhyi et al. 2012). *In vitro* studies have shown that chemotherapeutic agents, such as hydrogen peroxide (H_2O_2) and citric acid (CA), might be more suitable for removal of biofilm on titanium surfaces than CHX (Ntrouka et al. 2011a, Ntrouka et al. 2011b, Mouhyi et al. 2012). For mechanical cleaning of titanium implant surfaces the use of air-powder abrasive with either sodium bicarbonate or amino acid glycine powder seems to be promising. *In vitro* studies suggest that this method effectively cleans titanium surfaces of different surface topography (Louropoulou et al. 2014b), without adversely affecting its biocompatibility (Louropoulou et al. 2014a). Although hydrogen peroxide, citric acid and air-powder abrasive have been used for implant surface decontamination during surgical peri-implantitis treatment (Behneke et al. 2000, Khoury & Buchmann 2001, Leonhardt et al. 2003, Roos-Jansåker et al. 2007a, Roos-Jansåker et al. 2007b, Deppe et al. 2007, Duarte et al. 2009, Toma et al. 2014), so far no randomized controlled trials have been conducted evaluating exclusively the influence of these chemical agents/mechanical cleaning procedures on treatment outcomes. In addition, clinical safety and possible adverse effects of the materials/procedures need to be taken into account. For example, it has been described that air-powder abrasive could cause subcutaneous emphysema, which is a potential life-threatening complication (Bassetti et al. 2014).

In the studies described in chapters 6 and 7 no regenerative procedures were applied in conjunction with the access flap, removal of granulation tissue and implant surface decontamination. Instead, the peri-implant bone was slightly recontoured (*i.e.* elimination of sharp bony edges) to allow for pocket reduction and better adaptation of the soft tissues. Although there is some evidence that (radiographic) defect fill of peri-implantitis defects following surgical treatment modalities with concomitant bone and/or bone substitutes is possible to some extent (Renvert et al. 2012), there is great heterogeneity among the obtained results and there is not yet consensus which materials and methods should be used. One of the factors that seem to influence the clinical outcome following regenerative therapy is the peri-implant defect configuration (Schwarz et al. 2010). Regenerative therapy could be promising for four-walled defects, but seems unfavorable for defects with other configurations such as horizontal defects or dehiscences (Schwarz et al. 2010). Vice versa, it could be hypothesized that resective surgical therapy performs better in conjunction with horizontal bone defects than with deep, angular defects. It therefore seems advisable that future studies evaluating any type of surgical treatment of peri-implantitis report on the specific peri-implant defect configuration, in order to allow for comparison of treatment effects among different groups of defect configurations.
Another point of discussion in the treatment of peri-implantitis is the administration of pre-operative/post-operative systemic antibiotics. As there are currently no (randomized) controlled studies to demonstrate the benefit on the use of antibiotics, there is no scientific proof to support one approach over the other (Renvert et al. 2012, Van Winkelhoff 2012). Therefore, in the studies described in chapter 6 and 7 no systemic antibiotic therapy in conjunction with the surgical peri-implantitis treatment was ren-

dered. In general, the world-wide increasing problem of antibiotic resistance urges us to restrict the use of antibiotics to those patients that are expected to benefit from it. It has been shown that large inter-individual variation exist in periodontitis patients treated with antibiotics (Matarazzo et al. 2008, Silva et al. 2011, Guerrero et al. 2014) and therefore it is recommended that antibiotics are prescribed on an individual basis and possibly supported by microbiological information for optimal antibiotic choice and regime. This 'personal medicine approach' should also pertain to peri-implantitis patients, as it has been shown that peri-implantitis patients frequently yield submucosal bacterial pathogens resistant to therapeutic concentrations of clindamycin, amoxicillin, doxycycline and metronidazole (Rams et al. 2014).

Despite several treatment related factors one could think of many other factors that could determine the outcome of peri-implantitis treatment. The aim of the study described in chapter 8 was to identify such factors (prognostic indicators). For this, the datasets of the two studies described in chapters 6 and 7 were combined. This was possible because the research protocols of the two studies were nearly identical, patient groups were similar, treatments were performed by the same surgical team and the studies were conducted consecutively. It turned out that the outcome of the surgical peri-implantitis treatment was most significantly influenced by the amount of experience of the surgical team with that specific procedure. This 'learning effect' or phenomenon of improvements in performance over time when adopting a 'new' technique is well-known in medicine, psychology and engineering (Ramsay et al. 2000, Ramsay et al. 2002). Learning effects form an obstacle to undertaking and interpreting clinical trials and should be statistically considered in clinical studies (Cook et al. 2004). Additionally, it seems important that members of a surgical team are sufficiently trained and supervised when adopting a new technique and have a predefined level of experience with a specific procedure before participating in a clinical trial.
In addition to the factor 'experience of the surgical team', the mean amount of bone loss at baseline and smoking also proved to be strong prognostic indicators for the outcome of resective surgical peri-implantitis treatment. Less strong prognostic indicators were maximum pocket depth at baseline and presence of plaque during follow-up. Amount of bone loss and pocket depth at baseline can be seen as representatives for disease severity. Therefore, early diagnosis of peri-implantitis, which can be achieved by regular monitoring of the peri-implant tissues, seems crucial for achieving a successful peri-implantitis treatment outcome. Smoking and the presence of plaque are behavioral factors that can be altered by the patient and play a major role in development of peri-implantitis (Heitz-Mayfield 2008). As important part of their peri-implantitis treatment strategy, smokers should be encouraged to abandon smoking and all peri-implantitis patients should be encouraged and properly instructed to achieve high levels of oral hygiene.

Concluding remarks and future perspectives
The success percentages of the peri-implantitis treatment procedures evaluated in chapters 6 and 7 were rather low ('success' for 43% of the implants and 33% of the patients), indicating that it is difficult to treat peri-implantitis successfully, even by

a surgical treatment approach. This, combined with the notion that peri-implantitis seems to develop and progress faster than periodontitis, stresses the importance of disease prevention.

Prevention of peri-implantitis starts with maintenance of natural teeth and prevention of tooth loss. Dental implants should represent a 'last' resort and they should not replace teeth, but they should replace missing (or hopeless) teeth (Lang et al. 2007). Even compromised teeth that are treated and maintained regularly can yield high survival and success rates (Holm-Pedersen et al. 2007). It could be hypothesized that, in general, the advances in the field of oral implantology have made decisions on tooth extraction easier and consequently may have led to an increase in tooth extraction rate. Viewed from the perspective of peri-implant infections and the difficulties encountered in treating them successfully, this may not necessarily be a beneficial development.
In case of tooth loss, one should carefully decide on the most appropriate treatment option (conventional versus implant treatment), taking into account patient factors, potential risk factors, number of missing teeth, complexities (costs), related disadvantages of the treatment options and maintenance costs, amongst other factors (Lang et al. 2007, Albrektsson et al. 2012, Scheuber et al. 2012, Fardal & Grytten 2013). A careful individual risk assessment should be made prior to treatment. For this, a pre-implantological checklist, including all known risk factors for dental implant failure is necessary. Special attention should be given to factors such as general health, smoking, oral hygiene and (history of) periodontitis. Potential risk factors should be eliminated whenever possible and (periodontal) infection should be properly controlled before implant placement is considered. Extraction of teeth with a poor prognosis assists in reducing numbers of putative periodontal pathogens and total oral bacterial load and might limit the colonization of newly-placed dental implants with potentially virulent pathogens such as *P. gingivalis*. However, more (longitudinal) research is necessary to clarify the specific role of microbial factors in the development of peri-implant infections. The use of new, open-ended, molecular diagnostic techniques may allow for a better comparison between periodontal and peri-implant biofilms in both health and disease and may establish a better understanding of the preventive and therapeutic microbial implications (Faveri et al. 2015).

Placement of dental implants should be planned and executed appropriately, to eventually allow for a prosthetic reconstruction in harmony with the remaining dentition and accessible for proper oral hygiene measures (Serino & Ström 2009). In order to establish and maintain healthy peri-implant tissues, a preventive program (supportive therapy) should be initiated after implant installation, involving oral hygiene instructions and clinical examinations (Anner et al. 2010, Costa et al. 2012, Atieh et al. 2013). To allow for diagnosis of peri-implant disease and monitoring of disease progression clinical examinations should involve peri-implant probing and, when indicated, x-rays (Heitz-Mayfield 2008). Upon diagnosis of peri-implant disease, it is important to initiate a therapeutic intervention as soon as possible. The first step in a successful intervention represents the identification and, if possible, elimination of possible underly-

ing problems, such as cement remnants, improper fit of the prosthetic reconstruction, impossibility to clean the prosthetic reconstruction, implant malpositioning, overload or titanium intolerance. If the underlying problems can not be solved, it may be better to remove the implant.

Outcome of peri-implantitis treatment might not only be influenced by the chosen treatment strategy, but also by other factors such as experience of the surgical team, severity of the disease (bone loss and pocket depth) and behavioral factors (smoking, presence of plaque) (chapter 8). Increasing the knowledge on such prognostic indicators for outcome of peri-implantitis treatment is important, because it allows us to improve treatment strategies and, ultimately, improve treatment outcomes.
Although consensus exists that surgical therapy in addition to non-surgical therapy is most frequently needed to successfully treat peri-implantitis (Renvert et al. 2008, Esposito et al. 2012, Heitz-Mayfield & Mombelli 2014) still no consensus exist as to which protocol is most effective. This lack of a validated protocol exposes the urgent need for further research, preferable in the form of well-controlled clinical trials investigating only one treatment or one component of treatment at a time. Our research has shown that chlorhexidine rinsing of the implant surface, in addition to mechanical implant surface debridement, does not enhance clinical outcomes of resective peri-implantitis treatment (chapters 6 and 7). Therefore, future research should focus on other methods of implant surface debridement and decontamination. In addition, research should focus on the added value of systemic antibiotics in conjunction with a non-surgical and/or surgical treatment approach for peri-implantitis and on the indication for and added value of regenerative procedures.
Hopefully, the near future holds validated protocols for prevention and treatment of peri-implant infections and increase of the knowledge on peri-implant infections to such an extent that we no longer 'practice a lot more than we know'.

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