Peri-Implant Infections of Oral Biofilm Etiology

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Abstract

Biofilms are complex microbial communities that grow on various surfaces in nature. The oral micobiota tend to form polymicrobial biofilms, particularly on the hard mineralized surfaces of teeth, which may impact on oral health and disease. They can cause inflammation of the adjacent tooth-supporting (periodontal) tissues, leading to destructive periodontal disease and tooth loss. The emergence of osseointegrated dental implants as a restorative treatment option for replacing missing teeth has also brought along new artificial surfaces within the oral cavity, on which oral bacteria can form biofilms. As in the case of natural teeth, biofilms on implant surfaces may also trigger infection and cause inflammatory destruction of the peri-implant tissue (i.e. peri-implantitis). While there are strong similarities in the composition of the mixed microbial flora between periodontal and peri-implant infections, there are also a few distinctive differences. The immunological events underlying the pathogenesis of peri-implant infections are qualitatively similar, yet more extensive, compared to periodontal infections, resulting in a faster progression of tissue destruction. This chapter summarizes the current knowledge on the microbiology and immunology

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of peri-implant infections, including findings from the peri-implant crevicular fluid, the inflammatory exudate of the peri-implant tissue. Moreover, it discusses the diagnosis and current approaches for the treatment of oral infections.

4.1 Biofilms in the Oral Cavity

The oral cavity consists of both soft mucosal tissue surfaces and hard dental tissues all bathed in constantly secreted saliva. This anatomical niche of the human body constitutes a dynamic ecosystem that is continuously colonized by commensal microorganisms, which are collectively defined as the oral microbial flora. They have evolved along with the host, while their survival is tightly dependent on their capacity to use the available nutrients for their growth and their adaptability to the host's innate and adaptive immune system. It is estimated that the diversity of oral microbiota accounts for more than 700 different species, with at least 100 species populating the oral cavity of a given individual (Aas et al. 2005; Paster et al. 2001). The oral bacteria rarely grow in single planktonic form, but they naturally form biofilm communities with each other on the tooth surface. Biofilms exhibit a very high level of structural and functional bacterial organization, whereby the individual bacterial constituents communicate with each other by finely tuned molecular processes (also defined as "quorum sensing") (Huang et al. 2014). Biofilms demonstrate much more virulent characteristics compared to bacteria in planktonic state, as they exhibit altered gene expression patterns and are less penetrable by neutrophils, antibodies, or antimicrobial factors (Schaudinn et al. 2009), even by a factor of 500 (Costerton et al. 1995). Clinically, the "dental plaque" forming on the tooth surface holds the full properties of a biofilm (Marsh 2003).

Changes in the local microenvironment may cause shifts in the composition of the biofilm microflora, giving leeway to certain bacterial species to overgrow, enhance their virulence properties and eventually become opportunistic pathogens. Such species may be found at low numbers in health and can become pathogenic only when the newly established conditions permit them so. This is the principle drive for the "ecological plaque hypothesis", the predominant theory that explains the etiology of the polymicrobial oral diseases as a disturbance of the relationship between the resident oral microbiota and the response of the host that they populate (Marsh 2003; Marsh and Devine 2011). Dysbiotic biofilms can endure the host response and concomitantly exploit the inflammatory host response, in a manner that propagates the magnitude of the inflammatory tissue destruction, as is the case of bone loss in periodontitis (Hajishengallis and Lamont 2012; Hajishengallis 2014).

4.1.1 Biofilms and Oral Disease

Dental caries and periodontal diseases are the two main and highly prevalent oral diseases, both caused by biofilms growing on the tooth surface. Dental caries manifests essentially as the demineralization of the hard dental tissues (namely enamel and dentine), by acids generated due to the fermentation of dietary carbohydrates by the biofilms grown on the tooth surface. Its incidence has increased with sugar consumption and it is among the most prevalent infectious diseases in the industrialised world. If dental caries remains untreated, the biofilm-associated bacteria can eventually invade into the deeper soft dental pulp tissue, causing pulpitis, and subsequently tooth necrosis. Periodontal diseases are a major group of biofilm-associated oral diseases that destroy the tooth-supporting (periodontal) tissues as a result of excessive inflammatory response of the juxtaposed gingival tissue. Etiologically, they are

attributed to polymicrobial biofilms accumulated on the tooth surface, and particularly under the gingival margin (subgingival). The inflammation can be contained within the gingival tissue (gingivitis), and manifests as swelling and bleeding of the gingiva, symptoms that are easily identifiable by the patient. This condition affects virtually the whole global population, and is reversible once the biofilm is removed from the tooth surface and proper oral hygiene is instilled. Persistence and progression of an exacerbated inflammatory response can destroy the deeper periodontal tissues, namely the periodontal ligament that links the tooth surface to the supporting alveolar bone. The disease has now progressed to the stage of periodontitis which, apart from the loss of supporting bone and periodontal ligament, is also characterised by the formation of deep periodontal pockets. If left untreated, periodontitis will result in exfoliation of the affected tooth, impairing the chewing function and compromising the esthetic appearance. Periodontitis is the main cause of tooth loss in the industrialised world, and is perhaps the most prevalent inflammatory infectious disease in human adults, affecting approximately 1/3 of the population. One of the restorative treatment options for replacing teeth missing due to periodontitis is dental implants.

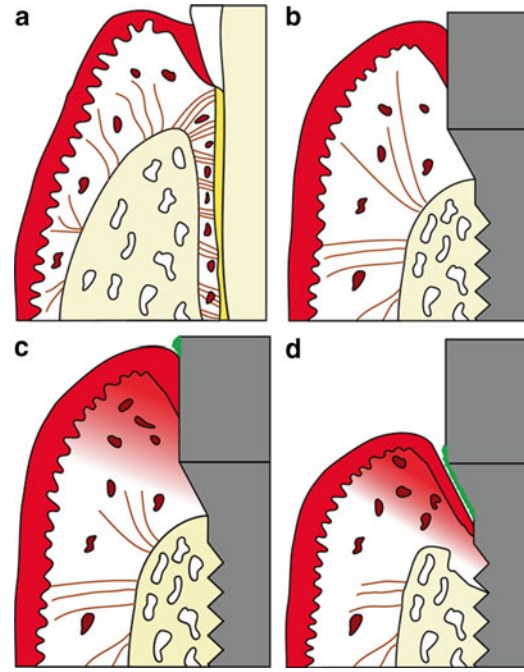

Fig. 4.1 Schematic representation of periodontal (**a**) and peri-implant tissues (**b**). The lack of periodontal ligament from the peri-implant tissues is evident. Accumulation of biofilm (marked *green*) on the implant surface will eventually result in inflammation of the peri-implant mucosa and establishment of peri-implant mucositis (**c**). Progression of this inflammation can lead to the destruction of the peri-implant tissues, including the supporting bone, culminating in peri-implantitis (**d**)

4.2 Dental Implants and Comparison to Natural Teeth

Dental implants are titanium-based screw-like devices that are surgically installed into the jaw bone, in the place of one or more missing teeth. Thereafter, a transmucosal abutment is adapted onto the implant, mediating the connection to the final prosthetic restoration that is visible in the patient's oral cavity. Hence, the patient's functional and esthetic needs are re-established. The titanium surface is biologically "accepted" by the surrounding bone tissue, and forms a connection known as "osseointegration". This titanium-bone relationship possesses the essential functional properties required to support the replacement of a missing tooth.

There is merit at this stage to define the main dissimilarities between periodontal and peri-implant tissues, in order to better understand peri-implant infections, or diseases (Fig. 4.1a, b). In the case of dental implants, the main difference with natural teeth is the absence of periodontal ligament, thus necessitating direct interface between the bone and the implant surface (Heitz-Mayfield and Lang 2010). Instead, the collagen fibers of the submucosal connective tissue are arranged parallel to the implant surface, thus forming a "collar". Consequently, the formed peri-implant crevice is deeper than the gingival crevice of natural teeth, resulting in a weaker physical barrier against bacterial invasion. Apart from very restricted mobility, the lack of the periodontal ligament also means restricted blood supply. Hence, the delivery of cells of the immune system, needed to tackle the early stages of

bacterial infection, is rather compromised. Collectively, these characteristics may render dental implants more susceptible to endogenous infection, than natural teeth. An exposed dental implant surface is also prone to microbial colonization. Thus biofilms can as well form on implants, with potential detrimental effects on the health of the surrounding peri-implant tissues.

4.3 Peri-Implant Infections: Classification and Diagnosis

Failures of dental implant function are classified either as early, or as late ones (Listgarten 1997; Tabanella et al. 2009). The early ones refer to incomplete osseointegration following surgical installation, and may be attributed to early loading, surgical contamination, poor compatibility of the material, or inefficient healing due to systemic disease. In late failures, the normal function of an already osseointegrated implant is disrupted, resulting from chronic infection of the peri-implant tissues.

Peri-implant mucositis is characterized by biofilm-induced inflammation localized on the soft peri-implant mucosa, but with no evidence of destruction of the supporting bone (Fig. 4.1c). Progression of the inflammation may lead to gradual destruction the bone, manifesting as peri-implantitis (Fig. 4.1d). Peri-implant mucositis and peri-implantitis are analogous to gingivitis and periodontitis of natural teeth (Heitz-Mayfield and Lang 2010). Mucositis occurs in approximately 80 % of patients with dental implants, and 50 % of the implants. The prevalence of peri-implantitis has varied reportedly from 28 to 56 % among patients, and 12 to 43 % among implants (Zitzmann and Berglundh 2008).

To-date the diagnosis of peri-implant diseases is based on clinical and radiographic criteria (Mombelli and Lang 1998; Kao et al. 1997). While mucositis is characterized by inflammation, erythema and bleeding of the tissue particularly during examination, peri-implantitis exhibits additionally a peri-implant pocket which is greater than 4 mm, potentially suppuration, and a characteristic "saucer-" or "crater-shaped" bone destruction around the implant, which is revealed radiographically (Heitz-Mayfield 2008). This biological complication around dental implants is characterized by profound inflammation of the tissues surrounding an implant in function with progressive loss of supporting bone (Lindhe and Meyle 2008). Peri-implantitis is becoming a pathological entity of growing concern among clinicians because of its aggressive pattern, and in certain cases rapid reach of the terminal stage i.e. implant loss.

4.4 Peri-Implantitis as a Biofilm-Initiated Disease

Despite the early introduction of the term "peri-implantitis" (Levignac 1965; Lindhe and Meyle 2008), the infectious nature of the disease was documented almost two decades later (Rams and Link 1983; Rams et al. 1991). Treatment with osseointegrated implants was introduced in fully edentulous patients, and in such individuals there was no biological rationale to consider post-osseointegration infections, since periodontitis-associated bacteria were to be automatically 'removed' from the oral cavity together with the extracted teeth. Late implant failure was explained during many years by overloading or excess loading. However, a recent systematic review (Naert et al. 2012) clarified that no association between overload and peri-implant bone loss could be found in the absence of peri-implant inflammation. Indeed, research should address critical questions with regard to the etiopathogenesis of peri-implantitis, which in turn would guide evidence-based treatment.

Given the non-shedding surface of the dental implant in the oral cavity, it is easy to understand why biofilm formation on the implant is an inadvertent process. The mouth provides not only a portal of entry for bacteria but also an inherent environment for bacterial colonization and growth. In a similar fashion to the tooth, by the time the dental implant is exposed in the oral cavity, it is covered by an acquired pellicle layer i.e. an organic stratum mainly consisting of proteins, glycoproteins and lipids. The pellicle triggers early bacterial colonization by providing receptors for the adhesins of specific species of oral

bacteria. Early colonizers are *Streptococcus* and *Actinomyces* species (Nakazato et al. 1989; Li et al. 2004) and create the preconditions for the accumulation of late-colonizing bacteria such as *Fusobacterium* and *Prevotella* species (Hannig 1997; Aas et al. 2005). The bacterial colonization of the surface starts already 30 min after implant insertion, and these early bacterial species can be found as part of the mixed biofilm community on the implant surface even several months later (Furst et al. 2007). The mature biofilm can eventually detach with dispersal and spread further, a critical stage for bacterial dissemination and consequent colonization of deeper tissue sites.

In terms of initial (i.e. 4 weeks) subgingival colonization, the frequency of detection of different species is similar between natural teeth and implants. Nevertheless, the colonization pattern on implants appears to be initially slower than on natural teeth (Quirynen et al. 2005), given the pristine surfaces of the implant and the lack of the desired indigenous microbiota. The bacterial composition of the biofilm formed on implants closely resembles that of the neighboring teeth (Botero et al. 2005; Salvi et al. 2008). This leads us to postulate that the oral microbial flora, and especially that of neighboring natural teeth, acts as a "reservoir" for the biofilms that build-up around implants.

4.4.1 Peri-Implant Microflora Resembling Periodontal Microflora

The peri-implant microflora in health consists mainly of Gram-positive cocci and non-motile bacilli, and a limited number Gram-negative anaerobic species, resembling gingival health (Mombelli et al. 1987; Bower et al. 1989). The switch to peri-implant mucositis is associated with increased presence of cocci, motile bacilli and spirochetes, comparable to gingivitis (Pontoriero et al. 1994). The transition to peri-implantitis is accompanied by emergence of Gram-negative, motile, and anaerobic species that are commonly found in periodontitis (Mombelli et al. 1987; Mombelli and Decaillet 2011). An interesting finding is that the microbial composition of peri-implant pockets in partially edentulous patients resembles that of the neighboring periodontal pockets, while the presence of *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* can be higher in peri-implantitis (Botero et al. 2005; Hultin et al. 2002; Shibli et al. 2008). Hence, the qualitative composition of the microbial flora of peri-implantitis-associated biofilms is in concordance with periodontitis. A representative microscopy image of a submucosal biofilm sample obtained from a site with peri-implantitis is provided in Fig. 4.2, whereby the diversity of morphotypes and taxa can be depicted. Finally, a finding of further interest is that some bacteria retrieved from peri-implantitis biofilms, most often *Prevotella intermedia/nigrescens* and *Streptococcus constellatus*, may display *in vitro* resistance to one or more standard antibiotic treatments (Rams et al. 2013).

4.4.2 Distinct Peri-Implant Microflora

By use of molecular techniques in microbiological analyses we have nowadays appreciated the breadth of microbial diversity in the subgingival/submucosal biofilms. Though it may sound logical that implants and neighboring teeth share similar microbiota since they share a similar ecological niche i.e. interdental space, emerging evidence suggests that they could be microbiologically distinct from each other (Kumar et al. 2012; Dabdoub et al. 2013; Heuer et al. 2012). By use of broad-range PCR techniques (Heuer et al. 2012) and pyrosequencing (Kumar et al. 2012; Dabdoub et al. 2013) it was demonstrated that the peri-implant microbiome was distinct from the periodontal microbiome. Given the distinct topography and immunological characteristics of the implant compared to the tooth, the two ecosystems could be regarded divergent. This could explain why teeth and implants may harbour diverse bacterial lineages.

A number of microorganisms have been identified in peri-implantitis that are less regularly detected in periodontitis. These include, but not

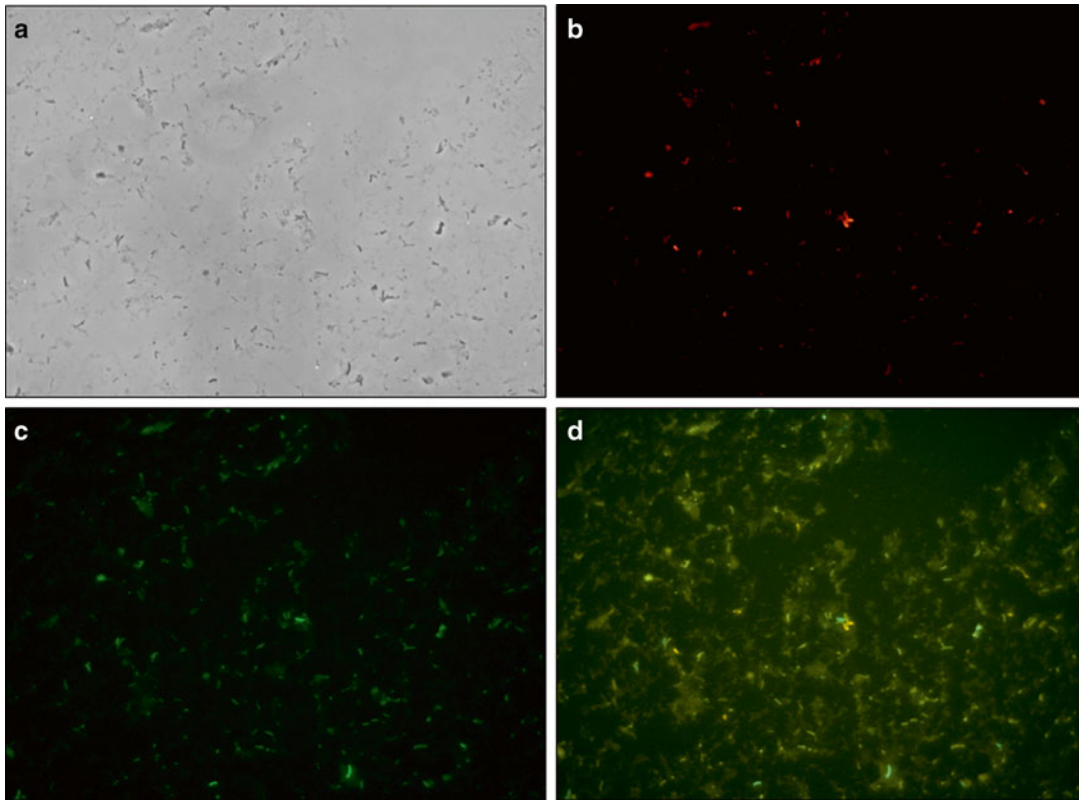

Fig. 4.2 Inverted light microscopy image of a submucosal biofilm sample obtained from a site with peri-implantitis (**a**). Epifluorescence microscopy image of the same field, combined with fluorescence *in situ* hybridization (FISH) using a 16S rRNA-targeted oligonucleotide probe for oral *Spirochaetes* (**b**), oral *Synergistetes* (**c**), or overlap of both (**d**)

restricted to, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacter aerogenes*, *Enterobacter cloace*, *Escherichia coli*, *Helicobacter pylori*, *Pseudomonas* spp, as well as *Candida* spp fungi (Renvert et al. 2007; Rams and Link 1983; Persson and Renvert 2014; Belibasakis 2014; Rams et al. 1991; Leonhardt et al. 1999, 2002, 2003). *S. aureus* is a versatile human pathogen discussed extensively in orthopedics as the leading etiologic agent of implant infection and of the associated osteomyelitis (Arciola 2009). It has a wide array of virulence factors, including up to 21 different adhesins or Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) and cytotoxins (Patti et al. 1994; Darouiche et al. 1997; Speziale et al. 2009). For this reason, it has attracted attention over recent years as a specific pathogen for peri-implantitis, distinct to periodontitis. The presence of *S. aureus* shortly after dental implant insertion can be confirmed even 1 year later (Salvi et al. 2008), while a recent microbiological study revealed by checkerboard methodology that significantly higher counts of *S. aureus* and *Staphylococcus anaerobius* were detected at implants with peri-implantitis than healthy implants (Persson and Renvert 2014).

Aerobic Gram negative bacilli (AGNB) include two wide and distinct categories: (i) bacteria that ferment lactose and belong to the large family *Enterobacteriaceae* (i.e. *E. coli. Enterobacter*, *Klebsiella*, *Citrobacter*), and (ii) non-enteric rods that do not ferment lactose (i.e. *Pseudomonas aeruginosa*, *Acinetobacter baumannii*). In a retrospective investigation of peri-implantitis cases (Charalampakis et al. 2012) culture analysis demonstrated the presence

of aerobic Gram-negative bacilli at moderately heavy or heavy growth in 18.6 % of patients with peri-implantitis. However, the microbial burden could not fully correspond to peri-implant disease severity. AGNB have been detected in previous studies both in peri-implantitis cases (Botero et al. 2005; Alcoforado et al. 1991; Leonhardt et al. 1999; Rosenberg et al. 1991), as well as in healthy implants (Nowzari et al. 2008b; Leonhardt et al. 1999; Nowzari et al. 2008a). Thus, the true role of AGNB in the etiology of peri-implant infections remains unclear.

4.4.3 Bacteria in the Implant-Abutment Interface

A bacterial leakage along the implant-abutment interface has been also discussed in the literature (do Nascimento et al. 2008, 2009; Gross et al. 1999; Callan et al. 2005). Given the fact that the interface includes microgaps ranging from 10 to 100 μm, we cannot exclude the scenario that microorganisms of 2 μm or less in diameter penetrate the passive fit between the implant components. Poor fit of attached components, inadequate torque, geometry of the implant platforms between the various implant systems, poor stability and micro-movements in the deeper inner portions of the system may enhance the extent of bacterial leakage (Binon 2000; Aloise et al. 2010). Such microgaps may function as 'nests' for anaerobic or microaerophilic bacteria to be protected form host defense mechanisms and persist for extended periods. However, the hermetic closure of a contaminated small compartment would serve as entombment, creating unfavorable conditions for bacteria to grow. Thus, the risk for peri-implant infection around two-part implant systems because of microbial leakage per se should be considered minimal.

4.4.4 Effect of Implant Surface on Biofilm

Rough implant surfaces were introduced in the dental market in order to enhance the rate of osseointegration. However, the implant surface roughness has a significant impact on the quantity and the quality of the plaque formed. A rough surface structure characterized by grooves and pits may provide the bacteria with 'protected' areas, inaccessible to conventional mechanical removal (Renvert et al. 2008). Other surface characteristics that enhance initial bacterial adhesion are high wetability and great surface free energy (Teughels et al. 2006). A recent *in vivo* study on the biofilm structure formed on titanium discs with different surface characteristics revealed different microbial patterns (Charalampakis et al. 2014). By SEM analysis it was demonstrated that the discs representing the moderately rough surfaces (Osseospeed™, TiOBlast™, Experimental surface) harbored a complex biofilm with tight intercellular bacterial bindings, whereas the discs with the turned surface hosted a biofilm that presented a pattern of spread bacteria forming less clusters. The study concluded that variations in the biofilm pattern may be associated with the different surface characteristics of titanium discs.

However, there is limited and contradictory evidence on the impact of implant surface on peri-implantitis. Some studies have found a positive correlation between smooth surface and peri-implant health (Astrand et al. 2004; Esposito et al. 2007), whereas others failed to find a correlation between type of implant surface and marginal bone loss (Gotfredsen and Karlsson 2001; Wennstrom et al. 2004). Nevertheless, it is also shown that surface characteristics of the abutments may not influence biofilm formation, or the extent and cellular composition of the inflammatory lesion (Zitzmann et al. 2002). Accordingly, no implant system or surface type was found to be superior in terms of marginal bone preservation (Abrahamsson and Berglundh 2009).

Last but not least, like in natural teeth, implant surfaces are immediately populated by salivary mucoproteins, which are required for the adhesion of bacteria (Kolenbrander et al. 2010). These are genetically defined in each individual, and may coat the surfaces of both natural teeth and implants, before being recognized

by the same bacterial species. It is therefore tempting to postulate that potential differences in bacterial adhesion due to surface microstructure may partially be equilibrated by the mediating salivary pellicle (Busscher et al. 2010). Hence, given the inevitable mediation of the pellicle in bacterial adhesion, implant surface characteristics may not notably affect the initial stages of biofilm formation.

4.5 Histopathological Events in Peri-Implant Infections

Like in the case of natural teeth, the development of a biofilm on the implant surface is an igniting factor of the inflammatory response of the surrounding peri-implant tissues. Peri-implant mucositis is characterized by inflammation that is confined to epithelium, connective tissue loss, microvascular changes (Sanz et al. 1990), and increased infiltration of leukocytes (Zitzmann et al. 2002, 2004). The sequence of inflammatory events that take place in peri-implant mucositis is similar to those in gingivitis, but potentially of a larger extent than gingivitis (Ericsson et al. 1992; Berglundh et al. 1991, 1992). The switch to peri-implantitis is accompanied by a further influx of inflammatory cells into the affected area of the peri-implant mucosa, that now expands to reach the bone tissue (Gualini and Berglundh 2003; Talarico et al. 1997; Lindhe et al. 1992), while a large number of osteoclasts form onto the bone surface and initiate bone resorption (Carcuac et al. 2013).

4.5.1 Immune Responses to Biofilm in Peri-Implant Infections

The histopathological events associated with peri-implant infections have been characterized over the past two decades. Nevertheless, there are still pending questions regarding the molecular regulatory events underlying these described processes. Peri-implant mucosal tissue biopsies, as well as inflammatory tissue exudates are suitable biological material to investigate in depth the molecular events associated with peri-implant diseases. In that respect, diseased tissue obtained from peri-implantitis sites is shown to exhibit higher expression of several mediators of inflammation, including pro-inflammatory cytokines interleukin (IL)-6, IL-8 and tumor necrosis factor (TNF)-α, compared to healthy or peri-implant mucositis sites (Venza et al. 2010; Konttinen et al. 2006; Duarte et al. 2009b). Although tissue biopsies can give an actual view of the undergoing molecular events within the tissue, the invasiveness of the collection process makes it almost impossible to use this material on a regular basis. In contrast, the collection process of the inflammatory exudate of the peri-implant tissues, namely the peri-implant crevicular fluid (PICF), is much simpler and non-invasive. This topic is discussed further in the next section.

4.5.2 Peri-Implant Crevicular Fluid as a Reservoir of Inflammatory Mediators

The PICF is the inflammatory exudate of the peri-implant sulcus or crevice, which is the tight anatomical depending formed between the implant surface and the peri-implant mucosa. This niche can be converted into a peri-implant pocket as peri-implantitis progresses, fostering a submucosal biofilm. Similarly to gingival crevicular fluid (GCF) which bathes natural teeth, PICF is the outcome of increased permeability of the vessels within the underlying connective tissue, as an inflammatory response to the growing biofilm. Although, the molecular characterization of PICF is at its infancy compared to GCF, it is already known to contain several serum and locally produced molecules, such as tissue breakdown products, inflammatory mediators and antibodies directed against the bacteria of the biofilm (Adonogianaki et al. 1995). Therefore, analysis of the PICF might be suitable to evaluate the inflammatory status of peri-implant tissues, in a quantitative manner (Kaklamanos and Tsalikis 2002; Belibasakis 2014).

In healthy peri-implant tissues, the diffusion of PICF is rather passive and slow. However, its

volume amount is significantly increased at a given site once biofilm-induced inflammation is established (i.e. peri-implant mucositis). Human experimentally induced peri-implant mucositis studies have elegantly showed that both PICF volume and protein content increases by the end of the 3-week period of plaque accumulation protocol (Salvi et al. 2012). Despite the strong clinical similarities between human experimental gingivitis and peri-implant mucositis, the latter is presented with a more pronounced inflammatory response to biofilm accumulation (Salvi et al. 2012; Pontoriero et al. 1994; Schierano et al. 2008). Since the composition of PICF is modified along with the histopathological changes during the course of progressive inflammation, its molecular analysis may support the early detection of clinically undetectable changes.

Pro-inflammatory cytokines have been the primary candidates to be investigated in PICF, due to their central role in triggering the inflammatory process, and the good amount of knowledge that already derives in studies on GCF. It was confirmed that higher concentrations of TNF-α, IL-17 and IL-1β are present in PICF collected from peri-implantitis-affected sites, compared to healthy controls (Ataoglu et al. 2002; Curtis et al. 1997; Darabi et al. 2013; Vieira et al. 2013; Severino et al. 2011), whereas the opposite was the case for anti-inflammatory cytokine IL-10 (Casado et al. 2013). More recently multiplex cytokine arrays have been applied in the analysis of PICF, allowing for a broader simultaneous screening of multiple inflammatory cytokines and chemokines. In one such study, 12 markers were assessed in both peri-implant health and disease, including granulocyte macrophage colony-stimulating factor (GM-CSF), IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, interferon (IFN)-γ and TNF-α (Fonseca et al. 2014). In line with previous findings, there were no differences with regards to IL-6, IL-8, IL-10 (Severino et al. 2011), whereas the levels of IL-1β were significantly higher at peri-implantitis, compared to mucositis sites. Further studies also showed that peri-implantitis treatment reduced the PICF levels of IL-1β (Bassetti et al. 2013) and TNF-α (de Mendonca et al. 2009; Duarte et al. 2009a). Studies have also demonstrated that single nucleotide polymorphisms (SNP) in the IL-1 gene may hold an increased risk for the development of peri-implantitis, particularly when combined with smoking (Andreiotelli et al. 2008; Bormann et al. 2010). However, the presence of a specific SNP does not necessarily translate into higher levels of IL-1β in PICF (Lachmann et al. 2007; Jansson et al. 2005; Melo et al. 2012). Although there is good evidence of the involvement of IL-1β as a crucial mediator of the host inflammatory response in peri-implant tissues (Murata et al. 2002; Salvi et al. 2010; Ataoglu et al. 2002), this needs to be complemented with further interventional studies, whereby IL-1β inhibitors are part of the treatment. Moreover, other members of the IL-1 family, such as IL-18, which may display differential regulation from IL-1β, should also be considered in PICF (Hamedi et al. 2009; Bostanci et al. 2009).

Matrix metalloproteinanses (MMPs) are proteolytic enzymes with a strongly documented role in collagen degradation in various disease processes, including periodontitis (Sorsa et al. 2006). Their involvement in peri-implant tissue destruction has also received attention, and a number of studies have demonstrated that MMP levels in PICF from peri-implantitis sites are elevated compared to healthy sites, and that their enzymatic activity increases with disease severity (Kivela-Rajamaki et al. 2003a, b; Ozcakir-Tomruk et al. 2012; Xu et al. 2008; Teronen et al. 1997), while successful treatment results in reduction (Wohlfahrt et al. 2014; Salvi et al. 2012). Moreover, studies to determine whether MMP-8 is useful prognostic marker for peri-implantitis have shown that sites with higher PICF levels of MMP-8 are at greater risk for progressive bone loss (Arakawa et al. 2012).

As bone resorption is the hallmark of peri-implantitis, the regulation of osteoclastogenesis and osteogenesis-associated markers have also been studied in PICF. Similarly to findings in GCF obtained from sites with periodontitis (Belibasakis and Bostanci 2012), there is increasing evidence showing the association of the receptor activator of nuclear factor kappaB

ligand (RANKL) – osteoprotegerin (OPG) system with the occurrence and severity of peri-implantitis (Arikan et al. 2008, 2011; Sarlati et al. 2010; Guncu et al. 2012; Duarte et al. 2009a, b; Rakic et al. 2013). A few studies looking into other markers of bone metabolism, such as osteocalcin, reported that these are higher in PICF from peri-implant mucositis compared to healthy sites, whereas no differences were observed between peri-implantitis and either mucositis or health. Hence, elevated levels of osteocalcin in PICF may reflect increased local bone turnover around implants, rather than severe bone resorption (Murata et al. 2002).

4.6 Treatment of Peri-Implant Infections

The development of peri-implantitis shows comparable features to the development of periodontitis (Heitz-Mayfield and Lang 2010). Clinical treatment of peri-implantitis is performed by various means and there is currently no consensus on an official standard of care. Therapy generally aims at the settlement of inflammatory peri-implant processes and the preservation of hard and soft tissues, as evaluated by reduced bleeding on probing and reduced probing depth or stable radiographic bone level, respectively (Heitz-Mayfield and Mombelli 2014). Four phases of treatment are suggested in order to enable the successful treatment of peri-implantitis: (1) pre-treatment phase (oral hygiene, prosthodontic aspects), (2) surgical access (mucoperiosteal flap, bone substitute with or without membranes), (3) post-operative anti-infective control (systemic antibiotics, chlorhexidine rinses), (4) maintenance care (3–6 months) (Heitz-Mayfield and Mombelli 2014).

Supportive periodontal therapy is seen as means to reduce the likelihood of an onset of peri-implantitis (Salvi and Zitzmann 2014). Presurgical therapy of peri-implantitis should include measures of oral hygiene. These mostly result in a reduction of mucositis, by targeting the disruption of the associated biofilm (Mishler and Shiau 2014). Also air abrasive powders and laser treatment have been applied for the reduction of biofilms on implant surfaces (Mishler and Shiau 2014; Schwarz et al. 2013). Further, the mechanical debridement of the implant surface should be performed, using instruments that cause little trauma to the surface, antiseptics and possibly antibiotics (Lang et al. 1997). Surgical therapy enables the debridement of granulation tissue within a peri-implant defect and the possible performance of an implantoplasty by diamond burs, smoothening implant threads and structured implant surfaces to a polished state. Different surface topographies show differences in their susceptibility for peri-implant inflammation. During surgical therapy, the mechanical implantoplasty modifies the surface state, depending on the implant material (e.g. titanium grade 4) and the prior surface treatment (e.g. sandblasted, acid etched). This causes differences in post-surgical peri-implant bone formation (Albouy et al. 2011).

An additional regenerative step may be the application of bone replacement substances with or without membranes, aiming at peri-implant hard tissue regeneration. The desired aim is the re-osseointegration of a previously biofilm-covered surface. This regenerative therapy may be influenced by the peri-implant defect morphology and the absence or presence of keratinized mucosa (Schwarz et al. 2010). The type of prosthodontic restoration also influences the surgery. The operative site can be easily assessed through the removal of screw-retained implant crowns. In case of cemented crowns, debridement and implantoplasty may be limited to the buccal and approximal areas. A meta-analysis of treatment outcomes identified four surgical procedures, namely (a) access flap and debridement, (b) surgical resection, (c) regeneration with bone grafts, and (d) guided bone regeneration. A reduction in probing depth of 2–3 mm and a mean 2 mm radiographic bone gain is described for regenerative procedures. Most of these analyses have follow-ups of 1–2 years (Chan et al. 2014). Outcomes for regenerative approaches are described to vary the most.

Following surgical therapy, the onset of oral hygiene measures by the patient is important for

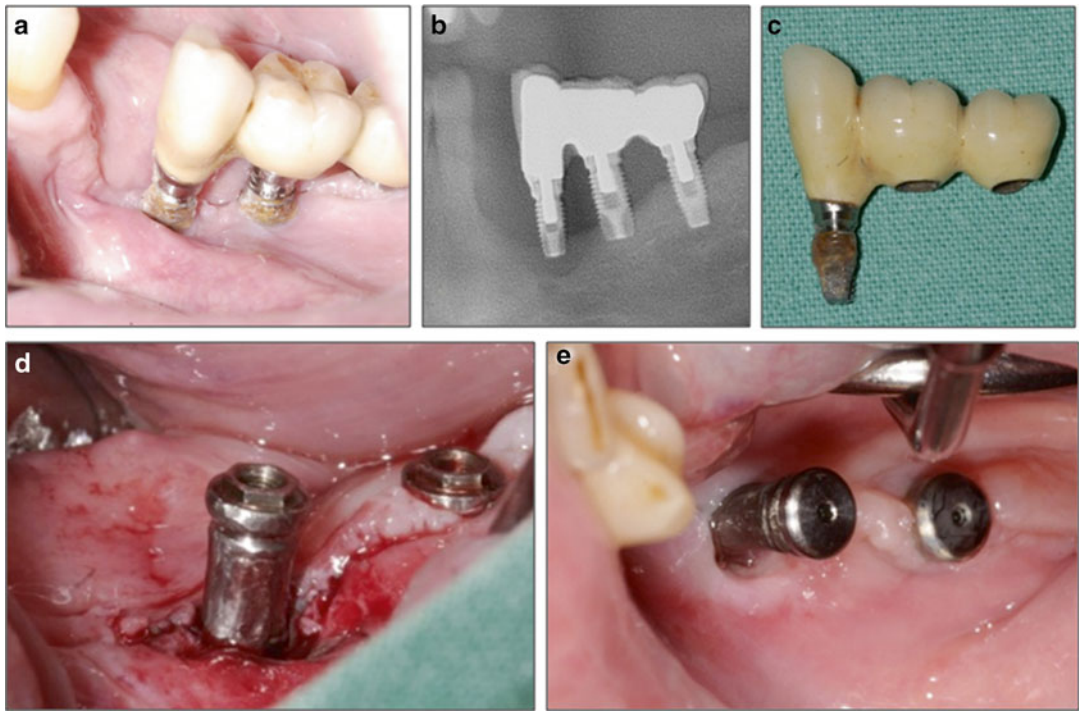

Fig. 4.3 Intraoral images of a patient with peri-implantitis. The implant threads are exposed to the oral cavity due to the destruction of the supporting bone. Large biofilm deposits have been accumulated over time on the implant surface (**a**). Accordingly, the radiographic image of the same site demonstrates severe bone loss around the implants. In fact, the implant on the left has no supporting bone at all (**b**). This implant was explanted (**c**), while the remaining two implants were maintained and underwent treatment that involved removal of the granulation tissue, implantoplasty and surface decontamination (**d**). The remission of inflammation and healing is evident already 2 weeks following the completion of the surgical treatment (**e**). Gingival healing caps were placed on the implants, and later on the prosthetic components were adapted, so that the implant function and esthetics are restored

long-term treatment success. It is important to maintain periodontal care over many years, as peri-implantitis may occur 5–10 years after implant placement and may reoccur at any time after treatment. Bone loss >2 mm post-prosthetic treatment and bleeding on probing could be indicators of peri-implantitis (Klinge 2012). A probing depth threshold of 5 mm without any bleeding on probing may predict the cessation of bone loss and the successful outcome of peri-implantitis therapy (Klinge 2012; Heitz-Mayfield and Mombelli 2014) In case of an onset of bone loss, implant mobility will follow. In such cases implant removal, debridement of the peri-implant defect, tissue regeneration and possibly re-implantation will follow. A clinical example of a peri-implantitis case is provided in Fig. 4.3.

4.7 Concluding Remarks on Peri-Implant Infections

It is evident that dental implant surfaces provide a suitable substrate for the growth of oral biofilms, in a similar manner as natural teeth. This is not without consequences, as the uncontrolled biofilm formation due to inefficient oral hygiene will eventually cause inflammation of the surrounding tissues in the form of peri-implant mucositis, and potentially lead to tissue destruction, manifesting as peri-implantitis. It is clear that the microbiota of the peri-implant biofilms derives from the various micro-ecological niches in the oral cavity, including the neighboring teeth, periodontal pockets, mucosal tissues and saliva. Although in principle the mixed microfolora of

peri-implant infections resembles that of periodontal ones, a number of non-typical oral taxa are more frequently found in peri-implantitis than periodontitis. Such are staphylococci, AGNB (e.g. enterobacteria and *Pseudomonas* spp) and *Candida* spp. The application of metagenomics in the analyses of biofilm samples is also likely to reveal specific microbial signatures in peri-implant infections. With regards to the pathogenesis of peri-implant infections the qualitative composition and sequence of the underlying immunological events resemble those of periodontal infections, but their magnitude is greater, thus resulting in a more aggressive progression of the disease. Hence peri-implant infections are "contemporary" infections caused by oral biofilms. They have emerged along with the continuous application of dental implants in restorative dentistry. While they are currently being treated in a similar philosophy as periodontal diseases, there is a need for reconsideration of their distinctive differences. This could lead to highly specialized therapeutic protocols, optimized for peri-implantitis.

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