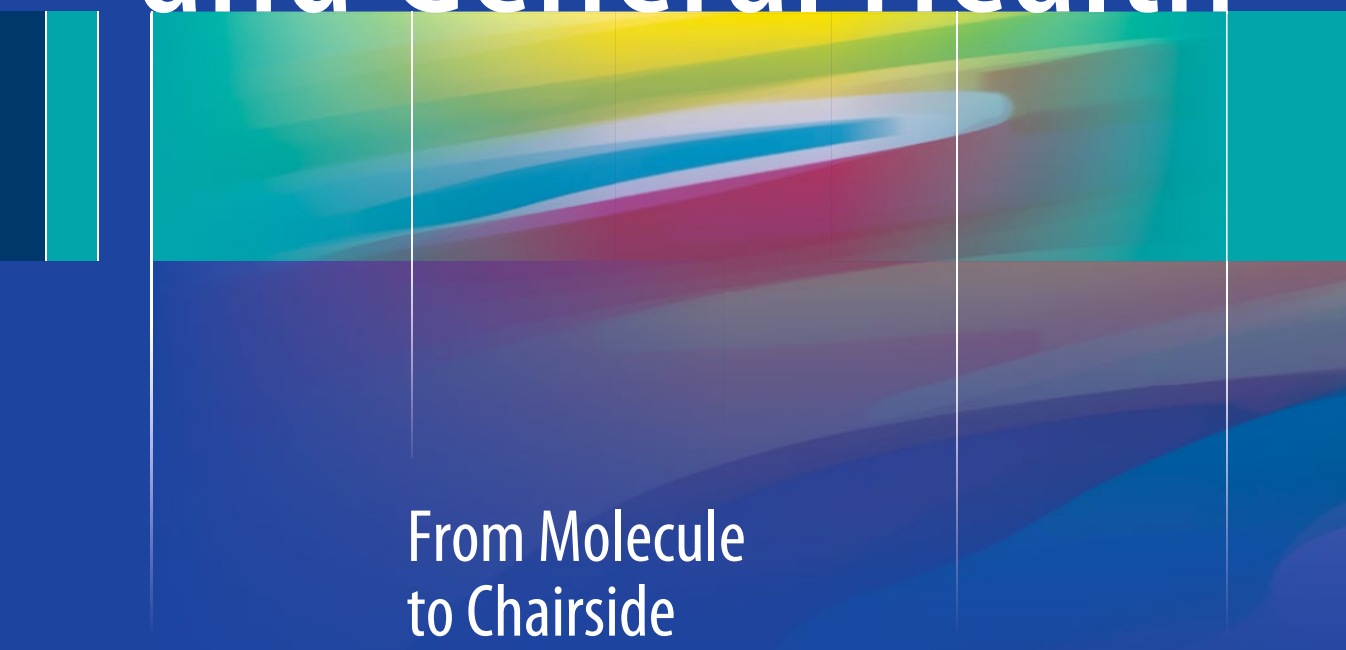
Anne Marie Lynge Pedersen
Editor

Oral Infections and General Health

From Molecule to Chairside



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About the Editor

Anne Marie Lynge Pedersen, DDS, PhD, is Associate Professor and Head of the Department of Odontology in the Faculty of Health and Medical Sciences, University of Copenhagen, Denmark. Dr. Pedersen graduated in 1992 from the Royal Dental College, Copenhagen, Denmark, and gained her PhD from the University of Copenhagen in 1997 for a thesis entitled *Salivary gland dysfunction in patients with primary Sjögren's syndrome*. She then joined the Section of Oral Physiology and Oral Pathology & Medicine, Department of Odontology, University of Copenhagen, as a research assistant, and progressed to become head of the department in 2013. From 2009 to 2012, Dr. Pedersen was Vice President and board member of the Scandinavian Division of the International Association of Dental Research (IADR), and she became President of the Division in January 2013. She has also served as an appointed board member of the Danish Dental Association Research Foundation (2007–2013) and acts as a consultant to the Danish Dental Association on topics such as xerostomia, salivary gland function, oral mucosal diseases, and pharmacology. Dr. Pedersen has been the organizer of national and international conferences, seminars, and symposia and is a referee for many leading international scientific journals.

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Part I

Background Topics

Introduction

1

Anne Marie Lynge Pedersen

Abstract

The idea for this book came about in relation to a symposium organized by the editor to present a research update that summarizes new findings related to the oral microbiota and the interaction between oral infections and general health. The symposium was held at the Annual Meeting of the IADR Continental European Division (CED) and Scandinavian Division (NOF) in Florence, Italy, in 2013. Consequently, the content of this book is largely based on contributions from European researchers within the field, but nonetheless the science presented in the various chapters embodies the global aspects of the topic and is therefore of significant relevance for researchers as well as health-care professionals throughout the whole world.

The healthy oral cavity is normally colonized by bacteria, fungi, and viruses. It has been estimated that more than 600 bacterial species colonize the oral cavity of which some may be pathogenic and others are symbiotic or commensal. The normal oral microbiota may be disrupted by a large number of factors including poor diet, malnutrition, poor oral hygiene, tobacco smoking and alcohol consumption, but also by several systemic diseases as well as the medications used for treating them, especially those associated with immunosuppression and/or salivary gland dysfunction. A disturbance of the balance between the oral microbiota and the host immune system results in a shift from a healthy state to a diseased state leading to inflammation and infections of the oral hard and/or soft tissues. The most common oral infectious diseases include dental caries, periodontal disease, and oral candidiasis.

Dental caries is one of the most prevalent diseases in humans. Even though the incidence of dental caries has decreased during the last decades, it is still a major problem in most industrialized countries as it affects 60–90 % of school-aged children and the vast majority of adults and obviously a larger problem in developing countries with poor living conditions and limited availability and accessibility of oral health services. In Chap. 2, Professor Twetman reviews

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a number of systemic diseases that are associated with an increased frequency of dental caries. As stated by the author, there are still obvious gaps of knowledge concerning the interaction between caries and general diseases which calls for further studies covering risk factors, associations, as well as cost-effective interventions.

Periodontitis is a serious oral infection, which is estimated to affect 15–20 % of the adult populations worldwide. Severe periodontitis may lead to premature tooth loss. Furthermore, most children and adolescents worldwide display signs of gingivitis. An increasing number of studies have suggested that oral infection, especially marginal and apical periodontitis, may affect the course and pathogenesis of a variety of clinically important systemic diseases. In Chap. 3, Professor Bruno Loos reviews current knowledge on the inflammatory mechanisms linking periodontal diseases to cardiovascular diseases. In the past decades a large number of studies have also demonstrated a linkage between periodontitis and diabetes mellitus. This linkage appears to be bidirectional. The proposed mechanisms or pathways by which these two diseases interact are reviewed by Professor Palle Holmstrup and Allan Flyvbjerg in Chap. 4. A possible two-way interrelationship has also been suggested for periodontitis and rheumatoid arthritis which is described in details by Professor Palle Holmstrup and Claus Nielsen in Chap. 5. Possible mechanisms behind the systemic effect include spreading of bacteria present in the periodontal pockets as a result of transient bacteremia and release of circulating oral microbial toxins and pro-inflammatory mediators caused by immunological injury induced by oral microorganisms. Insidious dental infections can worsen the condition and turn out to be life threatening in immunocompromised patients like recipients of kidney and liver organs. Consequently, oral infections should be diagnosed and properly treated before as well as during and after organ transplantation. Chap. 6 by Professor Jukka H. Meurman addresses the associations between dental infections and liver and renal diseases. According to WHO, the number of cancers is estimated to increase by 70 % by the year 2030 due to the aging of the populations. The prevalence of oral cancer is particularly high among men, and it is the eighth most common cancer worldwide. Recent research suggests that oral infections may play a role in the development of cancer. In Chap. 7, Professor Jukka H. Meurman reviews current knowledge on the mechanisms by which oral infections may influence the process of carcinogenesis.

Oral candidiasis is a common opportunistic infection usually caused by the overgrowth of *Candida* species. There are several predisposing to oral candidiasis including use of antibiotics, steroid inhalers or systemic steroids, high-carbohydrate diet, malnutrition, smoking, wearing dentures, and impaired salivary gland function. The prevalence of oral candidiasis is high in immunocompromised patients such as patients with HIV infection, Sjögren's syndrome, malignancies, and diabetes. Hence fungal infections are becoming a serious public health problem, particularly for the growing population of elderly people, immunocompromised patients, as well as patients with salivary gland dysfunction. Although the introduction of highly active antiretroviral therapy (HAART) has made oral candidiasis less common, HIV-associated oral lesions still remain significant with oral candidiasis as the most typical lesion. An update on oral candidiasis in medically compromised patients and the various current methods used to diagnose oral candidiasis, their advantages and disadvantages, as well as with new perspectives in using molecular techniques is given by Associate Professor Camilla Kragelund and coworkers in Chap. 8.

The last chapter in part II deals with the influence of salivary gland dysfunction, i.e., affection of the quantity and quality of saliva, on the occurrence of oral infections. In Chap. 9, Associate Professor Siri Beier Jensen and Anne Marie Lynge Pedersen review the most conditions associated with severe salivary gland dysfunction and their influence on oral health, i.e., Sjögren's syndrome, cancer therapy, and intake of medications.

Emerging knowledge on the oral microbiota challenges the current practice of chairside diagnostics. A number of new molecular techniques

are now used to analyze the microbiome in health and disease including HOMINGS, oligotyping, high-throughput sequencing, whole genome shotgun sequencing, single-cell genome sequencing, metatranscriptomics, and community-wide transcriptome analysis. Chap. 10, part III, by Professor Ingar Olson deals with the human oral microbiome which contains bacteria, bacteriophages/viruses, archaea, fungi, and protozoa, in health and common oral diseases. The salivary microbiota is a highly complex microbial community, containing oral microorganisms shed from various oral surfaces. Saliva can be easily and noninvasively collected, and compositional changes of the salivary microbiota may potentially serve as a biomarker used for screening of oral and systemic diseases as reviewed by Assistant Professor Daniel Belström in Chap. 11.

The final part of the book addresses recent research in treating or even preventing oral infections. The field of using probiotics is promising and may offer a novel approach of future handling oral functions as reviewed by Dr. Mette Rose Jørgensen and Associate Professor Mette K. Keller (Chap. 12). Also the management of patients with oral candidiasis is dealt with in this part of the book, in Chap. 13 by Associate Professor Camilla Kragelund and coworkers.

As we discover in this book, it seems justified to state that good oral health is important not only to prevent oral diseases but also to maintain good general health and vice versa.

Part II

Oral Infections and General Health

Dental Caries and General Health in Children and Adults

2

Svante Twetman

Abstract

Caries is a biofilm-mediated noncommunicable disease fueled by dietary sugar, neglected oral hygiene, and reduced saliva flow. General diseases may influence the oral environment through its pathogenesis, medication, and/or the caring of the condition. Associations between caries and chronic diseases are mainly derived from case–control studies with various sample sizes and quality of matching. Few observational studies are available and the majority of all research is conducted in childhood and among older adults. There is an increased caries risk for subjects with obesity, severe asthma, poorly controlled type 1 diabetes mellitus, and congenital heart diseases. An elevated caries frequency has also been reported for children with neuropsychiatric disorders and cleft lip palate and long-term cancer survivors. Frail elderly with cognitive impairments constitute a growing age group in society with caries risk due to age- and medication-induced salivary reduction. However, a general disease may not always have a negative influence on dental health. Therefore, a regular individual caries risk assessment is of utmost importance for clinical decision-making and tailoring of recall intervals. There is good evidence that preventive measures based on fluoride, saliva stimulation, and sugar awareness can prevent, control, and even arrest caries lesions in medically compromised patients of all ages.

2.1 Introduction

Dental caries is still the major cause of tooth loss, and the severe forms of the disease are associated with pain and feeding problems as well as impaired well-being and quality of life (Selwitz et al. 2007). In 2010, untreated caries in permanent teeth was the most prevalent chronic

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condition worldwide, affecting 2.4 billion people (Kassebaum et al. 2015). In addition, around 621 million children exhibited untreated caries in their deciduous teeth (10th most prevalent condition). With more than 45 % of the global population affected, it seems obvious that there is an overlap with general diseases. Interestingly, there was evidence that the current burden of untreated caries has shifted from children to adults, with three prevalence peaks at ages 6, 25, and 70 years (Kassebaum et al. 2015). Therefore, caries is a concern throughout the entire life course, and prevention and oral health promotion are needed for all age groups. This is especially important in the light of the current demographic trends with an increasing life expectancy and fewer non-dentate elderly persons. In general dental practice, around 30 % of all patients have medical issues that need to be considered, but an obvious concern is that medical data often are underreported in dental records; patients seem to regard such information as irrelevant for the dental care. The purpose of this chapter was to review the comorbidity between caries and the common general chronic diseases of which many have strong social and lifestyle components. The focus lies on children and elderly simply because there is a gap of studies and knowledge concerning young and middle-aged adults. The ambition was not to cover rare syndromes and conditions described in case reports. Furthermore, diseases with impact on dental erosion, the oral mucosa, and periodontal conditions are described elsewhere.

2.2 Link between Caries and General Diseases

Caries is a biofilm-mediated disease resulting from a complex interaction between the commensal microbiota, host susceptibility, and environmental factors such as diet (Wade 2013). The resident oral microbiota is normally diverse and beneficial to the host but the stability can be disrupted by stress. The repeated exposure of healthy biofilms to dietary sugars, and hence to low pH, favors the growth and metabolism of acid-producing and acid-tolerating bacteria (i.e., mutans streptococci, lactobacilli, bifidobacteria, *Scardovia*), causing dysbiosis (Marsh et al. 2014). Thus, caries is not a classical infection but should be regarded and handled as a noncommunicable disease. From a chemical point of view, caries is an imbalance between mineral loss and mineral gain; when the loss is dominating over time, a caries lesion eventually becomes visible. In a simplified way, demineralization occurs at low pH conditions in the oral biofilm (plaque) and remineralization at pH levels around neutral and above. It is therefore important to understand that any medical condition, medication, or behavior that directly or indirectly affects the pH stability in the oral environment and favors the overgrowth of aciduric species can be linked to caries. One common example is the use of xerogenic drugs which reduce oral clearance and prolong an acidic environment. A systematic review of literature has concluded that medication-induced salivary gland dysfunction (MISGD) constitutes a significant burden in many patients and may be associated with important negative implications for oral health (Aliko et al. 2015). Radiation therapy in connection with head and neck cancer is also associated with xerostomia and hyposalivation and rampant caries development at any age (Jensen et al. 2003). Another common example is intellectual or cognitive impairment among elderly that negatively affects the ability to maintain a proper oral hygiene. On top of the direct influence of the disease and its medication, the caring involved with the disease may increase caries risk. It is especially true in childhood, when the onset of a chronic disease is stressful for the whole family. The oral hygiene can be put aside for more "urgent" problems, and compensation through sweets and candies are often used to comfort the child.

To summarize the paragraph above, caries can be linked to any medical or behavioral condition associated with sugar behavior, polypharmacy, and impaired ability to regular cleaning. The most vulnerable groups seem to be young children and frail elderly, although the onset of a general chronic disease may affect the caries burden at any age. It should however be underlined that a

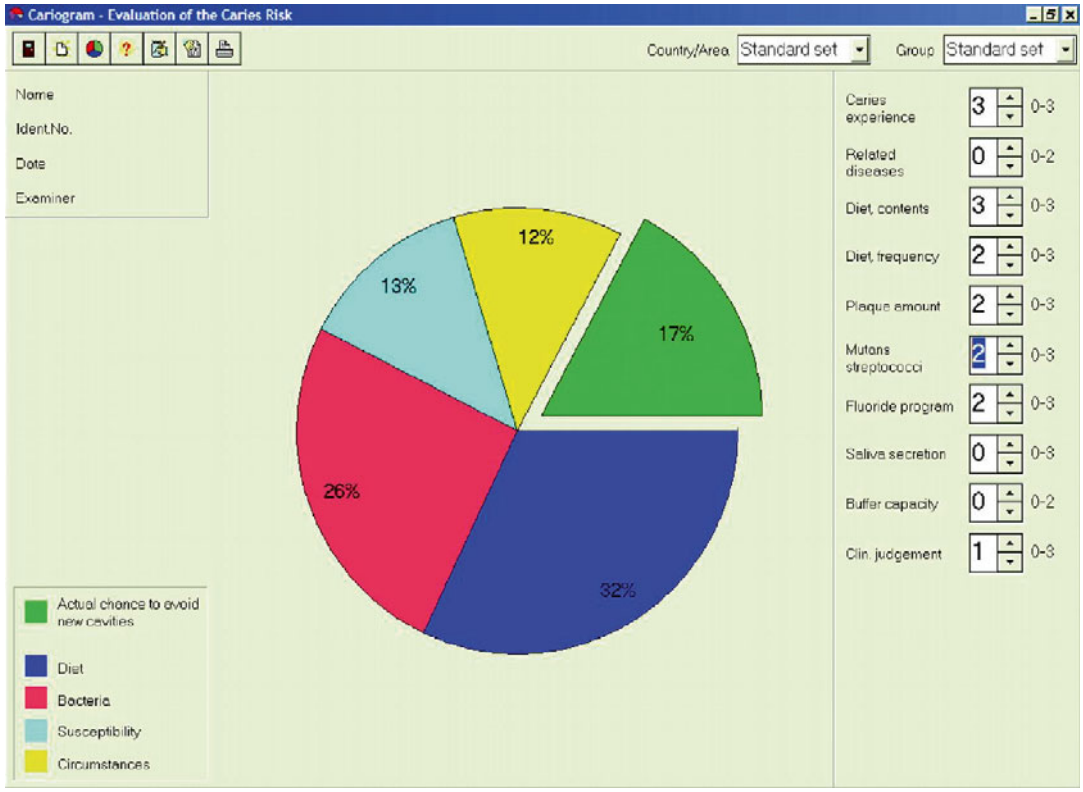

Fig. 2.1 Example of a computer-based caries risk model (Cariogram). After input, the computer is weighting preselected algorithms on bacteria, oral hygiene, circumstances, and susceptibility toward each other. The *green sector* indicates that this patient only has 17 % chance to avoid new caries in the near future and that the major cause is a sugar-rich diet (*blue sector*). The program is interactive for patient education and motivation. It can be downloaded free of charge in many different languages at www.mah.se

disease as such, with few exceptions like Sjögren's disease, will not necessarily ruin the dental health within a certain time period. Therefore, an *individual* caries risk assessment should always be performed as an integrated part of the clinical decision-making. There is evidence to suggest that comprehensive risk models are more accurate than the use of single factors, albeit no existing model performs superior to another (Mejàre et al. 2014). The use of structured templates or computer-based software (Fig. 2.1) is regarded as best clinical practice due to its consistency and didactic values (Twetman et al. 2013). The risk assessment should be repeated periodically throughout life and especially when life events occur, such as the onset of a chronic disease (Twetman et al. 2013). The outcome of these assessments should ideally form the basis for subsequent and tailored preventive and/or restorative treatment.

2.3 Common Research Designs

There are two principal study designs utilized to investigate the relationship between general diseases and dental caries; case–control and observational studies (Fig. 2.2a, b). The case–control approach is most common and starts with the selection of subjects with a defined disease (cases) and subjects without this disease (controls). The prevalence of caries can be compared in a cross-sectional way, and explanatory variables, or factors related to both the general disease and caries, can be collected retrospectively through a review of medical and dental records.

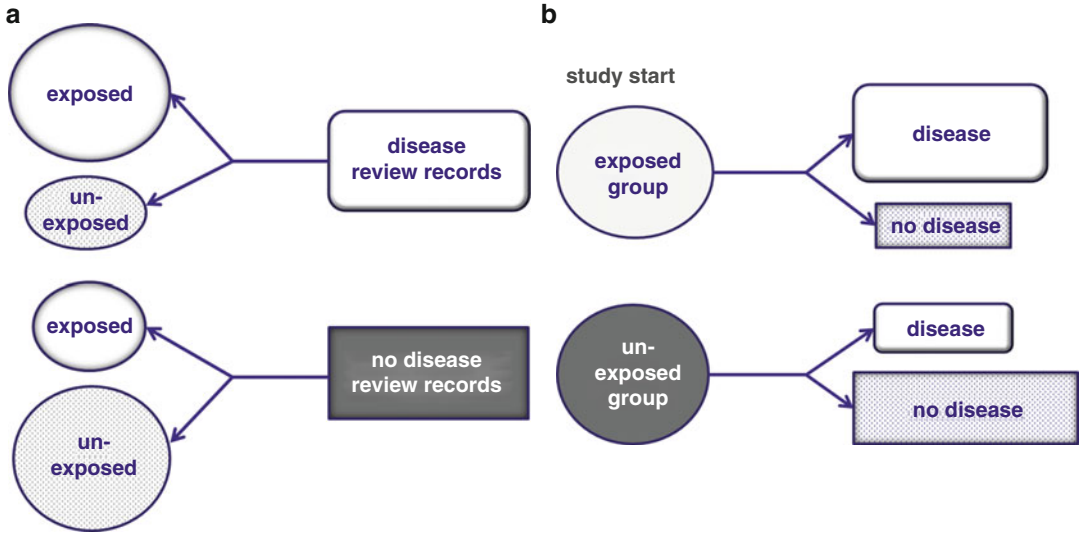

Fig. 2.2 (**a**) A case-control study begins by selecting subjects with disease (cases) or no disease (controls). (**b**) An observational study begins with exposure and the outcome is registered by time

This approach is easy to implement but the quality is strongly dependent on the matching process and the validity of the "historical" registrations. In a trustworthy study, the cases and the controls must be carefully matched for sex, age, socioeconomic and educational background, cultural norms, and lifestyle. The fact that case–control studies many times are presenting conflicting results is likely due to inadequate matching. Other problems that may flaw the conclusions are small sample sizes and diverging disease definitions for the "cases." From an evidence point of view, the prospective observational study design is more robust and controllable for the researcher but requires a considerable budget and time; a large number of patients must be included and followed for years with periodical reexaminations. The major threat for reliable conclusions is a large attrition rate (lost to follow-up) and/or a low incidence rate of the disease under study. A power calculation must therefore be conducted prior to the study in order to include a proper number of subjects. From an ethical point of view, it is equally problematic to run a project with too few persons as it is to enroll too many in demanding examinations and samplings. A mix of the abovementioned study designs is the nested case–control approach in which only a few defined cases and controls are selected from the full cohort. The main advantage is that not all patients must be sampled or examined over time which keeps down the workload and costs for an otherwise costly and resource-demanding trial.

2.4 Obesity/Overweight

Obesity and overweight is a growing problem among children and adults worldwide. The etiology is complex but overeating and calorie-rich diets are common compartments. A frequent intake of sucrose-containing food and beverages may be detrimental for the dental health (Arola et al. 2009). The relationship between sugar and caries is however not as strong today as it was decades ago which is commonly explained by the widespread access to fluoride in water and toothpaste. From the evidence perspective, it is beyond doubt that fluoride exposure is the key element in caries prevention and caries arrest at all ages throughout life (Griffin et al. 2007; Twetman and Dhar 2015). The link between caries and overweight in case–control trials is somewhat controversial and highly dependent on inclusion criteria and matching. Even systematic reviews have come to diverging conclusions; one established a significant

relationship (OR = 3.7) between obesity and dental caries in children from industrialized, but not from newly industrialized countries (Hayden et al. 2013), while another failed to note any relationship between overweight and caries burden (Silva et al. 2013). A fact that partly can explain the different conclusions is that dental caries may be associated with both high and low body mass index (Hooley et al. 2012). Unfortunately, studies in adults and elderly are largely lacking, but obviously diet recommendations and restrictions in order to reduce weight and prevent caries go hand in hand. Obesity is a part of the metabolic syndrome, and according to the common risk factor approach, dentists, together with all other health professionals, should embrace the current WHO guidelines and motivate their patients to reduce the free sugar intake to less than 10 % of the total energy intake (Moynihan and Kelly 2014). Free sugars include monosaccharides and disaccharides added to foods by the manufacturer, cook, or consumer, and sugars naturally present in honey, syrups, fruit juices, and fruit juice concentrates. A further reduction to below 5 % of total energy intake, or roughly 25 g (six teaspoons) per day, would provide additional health benefits.

2.5 Asthma

Asthma affects 6–8 % of the population and may influence the oral ecology through behavioral and medical pathways. Severe asthma is often associated with dry mouth, thirst, and frequent wake-up periods at night when sugar-rich beverages and fruit juices must be avoided. Furthermore, the steroid-containing inhalators, as well as beta-2 agonists, may have low pH values which favor the growth of acid-tolerating phenotypes in the oral biofilm, and increased levels of mutans streptococci are commonly unveiled in the saliva of asthmatic children (Alaki et al. 2013). Although there are conflicting reports on the relationship between asthma and dental caries in the literature (Maupomé et al. 2010), a meta-analysis has suggested that asthma doubles the risk of caries in both primary and permanent dentition (Alavaikko et al. 2011). Based on 11 studies, the odds ratio was 2.7 in the primary dentition, and the corresponding value in the permanent dentition was 2.0 based on 14 studies (Alavaikko et al. 2011). It was concluded that physicians and dentists should reconsider preventive measures against caries for persons with severe asthma and strongly recommend water rinses immediately after the use of inhalators.

2.6 Diabetes

Diabetes can affect the stability and profile of the oral biofilm through frequent meals and an increased output and leakage of glucose in saliva and gingival crevicular fluid. In the past, when the management of diabetes mellitus in childhood basically relied on slow-acting insulin and a highly restricted diet, subjects with diabetes exhibited less caries than a non-diseased population. With today's continuous monitoring of glucose, rapid-acting insulin, or insulin pumps, the type 1 diabetic child can live a more or less normal life with a less restricted diet. Furthermore, the oral health awareness among diabetics has increased in recent years with its close link to periodontal problems. Consequently, the results from case–control studies with diabetic patients have therefore slightly changed over time. According to recent systematic reviews, there is no consistent relationship between type 1 diabetes mellitus (T1DM) and dental caries in childhood (Ismail et al. 2015), although patients with uncontrolled T1DM and poor oral hygiene may present increased prevalence of dental caries (Sampaio et al. 2011). It is therefore important to collect information on the patient's recent HbA1c status; values above 8 % may indicate a poor compliance and may be associated with active caries development in schoolchildren and adolescents (Twetman et al. 2002). Concerning type 2 diabetes mellitus, no impact on the prevalence of dental caries has been reported (Sampaio et al. 2011).

2.7 Congenital Heart Disease

Congenital heart disease affects around 1 % of all children, and the condition is commonly associated with oral health problems. This has been

explained by an increased meal frequency, use of diuretic sucrose-containing medication, and frequent episodes of antibiotic treatment (Hansson et al. 2012). In addition, enamel defects and hypomineralization are prevalent in children with congenital heart disease which may predispose to caries development. Parental anxiety and overcompensation with sweets are psychological and behavioral factors often involved in the management of critically ill children. A case–control study has shown that children with congenital heart disease have three times more caries than healthy controls in spite of more prevention (Stecksén-Blicks et al. 2004). In the same study, a positive relationship between caries and the duration of the digoxin medication was established. Thus, a dental home should be established for children with congenital heart disease at an early age in order to implement individual treatment plans with frequent checkups during childhood.

2.8 Cancers

Long-term survivors of malignant conditions are subjected to long-term effects on oral health due to aggressive treatment protocols based on chemotherapy and radiation (Kaste et al. 2009; Effinger et al. 2014). In particular, the radiation therapy affects the salivary gland functions in a permanent or transient way, depending on location and radiation exposure. Consequently, patients who were post-radiotherapy exhibited higher DMFT values (decayed, missing, filled permanent teeth) compared to those who were post-chemotherapy and healthy controls (Hong et al. 2010). There is also data suggesting that children with leukemia displayed more caries than hospitalized children without cancer (Willershausen et al. 1998). However, among children that were caries-free at the onset of leukemia and displayed a low caries risk, the vast majority (87 %) were still unaffected after 3 years (Pajari et al. 2001). This picture seems to be less clear-cut among adults; a study has suggested an inverse relationship between head and neck squamous cell carcinoma and dental caries in a case–control study, although age and social factors may have played a role (Tezal et al. 2013). Nevertheless, patients with cancers should be considered at risk and candidates for saliva-stimulating measures and high-fluoride supplements.

2.9 Cleft Lip Palate

Children with cleft lip palate are often claimed to be caries prone in their maxillary incisors due to compromised tooth brushing during the infant period (Hasslöf and Twetman 2007; Antonarakis et al. 2013). An elevated prevalence of enamel defects may also contribute to caries susceptibility (Sundell et al. 2015). Consequently, it has recently been reported that children with cleft lip palate have more caries in the primary but not in the young permanent dentition compared to non-cleft controls (Sundell et al. 2015). There was however no clear association to the type or localization of the clefts. The increased caries risk must be taken into account by all members of the multi-professional team involved in the management of children with this syndrome.

2.10 Neuropsychiatric Disorders

Neuropsychiatric disorders in childhood may affect the possibilities to conduct a regular and proper oral hygiene. For example, studies in children with ADHD (attention deficit hyperactivity disorder) have suggested a 12-time increased risk for high DMFT values compared to controls that were matched concerning age, gender, ethnicity, and socio-economy (Broadbent et al. 2004, Blomqvist et al. 2011). The findings were adjusted for fluoride exposure, medical problems, diet, and oral hygiene. The neuropsychiatric disorders are however highly diverse and each family/child is unique, so the caries risk must be assessed individually and followed by targeted preventive measures.

2.11 Aging and Cognitive Impairment

There is no evidence to suggest a link between caries and healthy and vital older persons. However, for those with progressive intellectual

disabilities and dementia, oral health can rapidly be jeopardized, and root caries development is an atypical and increasing problem (Fiske et al. 2006; Anders and Davis 2010). This is also true for subjects with mental illnesses (Kisely et al. 2011). The main reasons are difficulties to clean and polypharmacy. Alzheimer's and Parkinson's disease are common examples on non-regular or sporadic oral cleaning and loss of ability to clean. Saliva plays a crucial role in maintaining oral health through its mechanical clearance, buffering effect, and being a source of mucins, immunoglobulins, enzymes, and antibacterial agents (Sreebny 2000). Increasing age means an increasing number of prescribed drugs that when combined in a "cocktail" very well can be xerogenic. Even a modest reduction of the unstimulated saliva secretion rate can have a strong impact on root caries development. It is therefore important to assess salivary gland function in elderly people and especially in frail elderly with diminishing autonomy. A sialometry can be helpful for motivating the patient to benefit from saliva-stimulating measures.

2.12 Caries Management

Caries prevention and management of the medically compromised patient span from community measures based on the common risk approach to tertiary prevention and treatment under general anesthesia. Although there is a palette of technologies for intervention, the core must be focused on evidence-based methods for primary prevention and noninvasive methods for secondary prevention. Examples of suitable strategies are presented in Table 2.1 and some practice points are listed in Box 2.1. It is important to keep in mind that there is no "one-size-fits-all" and that no method works better than its compliance. The patient's wish and demands must be considered in order to find and suggest the optimal care for each situation. For example, there are alternative ways to bring in fluoride to the tooth–biofilm interspace on a daily basis; the quality of evidence is varying, but a method with low evidence and good compliance may definitely be preferred over a measure with strong evidence but poor compliance.

Table 2.1 Examples of caries preventive measures suitable for the medically compromised patient

Measure	Age	Notes	Quality of evidence[a]
Self-applied measures			
Fluoride toothpaste	All ages	at least 1000 ppm F, twice daily	⊕⊕⊕⊕
High-fluoride toothpaste	>12 years	>1500 ppm F, biofilm metabolic inhibitor, high-risk subjects, root caries arrest	⊕⊕OO
Fluoride mouth rinse	>6 years	Daily 0.05 % NaF, post-brushing	⊕⊕OO
Fluoride chewing gum	>6 years	Saliva stimulation, remineralization	⊕OOO
Xylitol chewing gum	All ages	Saliva stimulation, biofilm metabolic inhibitor	⊕⊕OO
Professional measures			
Fluoride varnish	All ages	2–4 times per year, sustained fluoride release	⊕⊕⊕O
Silver diamine fluoride	All ages	44,000 ppm, lesion arrest, black-staining	⊕⊕OO
Fissure sealants	6–14 years	Prevention and noninvasive occlusal treatment	⊕⊕⊕O
Resin infiltration	Perm. teeth	Interdental non-cavitated lesions	⊕⊕OO
Professional tooth cleaning	All ages	Continuously repeated	⊕OOO

[a]Quality of evidence according to GRADE (Guyatt et al. 2011): *high* (⊕⊕⊕⊕) = based on high or moderate quality studies containing no factors that weaken the overall judgment; *moderate* (⊕⊕⊕O) = based on high or moderate quality studies containing isolated factors that weaken the overall judgment; *low* (⊕⊕OO) = based on high or moderate quality studies containing factors that weaken the overall judgment; *very low* (⊕OOO) = the evidence base is insufficient when scientific evidence is lacking, quality of available studies is poor, or studies of similar quality are contradictory

Box 2.1 Practice Points

- At least 3/10 of all adults have medical issues to be considered for oral health.
- Patient's health declaration and medicine list must be continuously updated and reevaluated.
- Patients/parents are often well informed and "experts" on their own disease – listen to them!
- Ask for permission to consult and discuss with the patient's physician when needed.
- A 2–3-day logbook is practical and valid to capture dietary habits, but a selective underreporting of food with high content of sugar is common.
- A powered toothbrush with rotating head is more effective than a manual toothbrush in reducing plaque and may facilitate oral hygiene for patients with reduced dexterity.
- Xerostomia is not equal to hyposalivation – the assessment of saliva function should include both serous and mucous glands as well as the patient's perception.
- Children with chronic diseases may have bad previous experiences and need "tell–show–do" learning to prevent behavior management problems.

Conclusions

Although direct causal associations sometimes are lacking, many general chronic diseases share the same risk factors (social, behavioral, cultural) with dental caries. Clinicians must be aware that any disease or medication that prolongs low pH conditions in the oral cavity is the driving force for biofilm dysbiosis that eventually will lead to impaired oral health and caries lesions. An individual risk assessment is therefore needed in order to tailor decision-making, prevention, and management to each unique subject, and this risk assessment must be updated regularly. In spite of extensive research, there are still obvious gaps of knowledge concerning caries and general diseases which calls for well-conducted studies covering risk factors, associations, as well as cost-effective interventions.

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3 Plausible Mechanisms Explaining the Association of Periodontitis with Cardiovascular Diseases

Bruno G. Loos, Wijnand J. Teeuw, and Elena A. Nicu

Abstract

The association between periodontitis and cardiovascular diseases is now well established. Cardiovascular diseases include atherosclerosis, coronary heart (artery) disease, cerebrovascular disease, and peripheral artery disease. Atherosclerosis is the underlying pathology of cardiovascular diseases. In this chapter, we describe plausible mechanisms to explain the link between periodontitis, atherosclerosis, and the subsequent cardiovascular diseases. The explanations for the development and exacerbation of atherosclerotic plaques in periodontitis patients include: (1) bacteremia, (2) a pro-inflammatory state, (3) a prothrombotic state, (4) an overactive immunity, (5) dyslipidemia, and (6) common genetic risk factors. Most likely, these plausible mechanisms play all simultaneously a role. Obviously, much more fundamental and clinic research is needed to further study the associations between periodontitis and atherosclerotic diseases.

3.1 Introduction

In the last three decades, the association between periodontal diseases and cardiovascular diseases has become apparent. The number of scientific publications and reviews on this topic has exponentially increased in this period. Since the landmark "Editorial" by Friedewald et al. in 2009 which appeared simultaneously in the *American Journal of Cardiology* and the *Journal of Periodontology* (Friedewald et al. 2009), and which confirmed the existence of the relationship between these two types of conditions, only further evidence has emerged. A joint workshop organized by the European Federation of Periodontology (EFP) and the American Academy of Periodontology (AAP) on the broad topic "the oral health-systemic health connection" further confirmed the epidemiological associations between periodontitis and cardiovascular diseases (Tonetti et al. 2013).

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Cardiovascular diseases include atherosclerosis, coronary heart (artery) disease, cerebrovascular disease, peripheral artery disease, and some other less frequent conditions. Atherosclerosis is the underlying pathology of cardiovascular diseases. In the framework of this chapter, where we describe plausible mechanisms to explain the link between cardiovascular diseases and periodontal diseases, we therefore will refer mainly to a possible role of periodontitis in atherogenesis and atherosclerosis and its (acute) consequences.

While the epidemiological associations between periodontitis and atherosclerosis and acute atherosclerotic events are now beyond any doubt, biological explanations on how the relationship may exist are not clear. This chapter describes possible and plausible mechanisms on how these two diseases might be linked. In this, we follow the consensus report on potential inflammatory mechanisms linking periodontal diseases to atherosclerosis and its consequences (Tonetti et al. 2013; Schenkein and Loos 2013).

3.2 Definitions of the Conditions

3.2.1 Periodontitis

Periodontal diseases include gingivitis, periodontitis, acute necrotizing ulcerative gingivitis or periodontitis, and periodontal abscesses. Specifically, periodontitis has been associated with atherosclerosis and acute atherosclerotic events. Periodontitis is a chronic inflammatory disease of the supporting tissues around the roots of the teeth. This disease results in the irreversible destruction of periodontal ligament and alveolar bone. The gingival tissues are highly inflamed and the periodontal connective tissues are infiltrated with leukocytes; the inflammatory reactions are actually at the base of loss of collagen and an increase in the number and size of blood vessels, giving the gingiva its swollen and reddish appearance. Over a period of years of chronic inflammation, a gradual loss of alveolar bone and periodontal ligament is apparent. If left untreated, teeth become mobile, may migrate, and eventually will exfoliate.

Traditionally, we differentiate between two types of periodontitis: aggressive periodontitis and chronic periodontitis (Armitage 1999). Severe periodontitis occurs in about 8–15 % of the population (Demmer and Papapanou 2010) depending on the definitions used for severe periodontitis and depending on the specific study population subjected to epidemiological studies. In countries with a high availability of dental care, with dental and health awareness, and with preventive measures available, the prevalence of severe periodontitis may be <10 %, while in countries with no dental care, the prevalence can even be >15 %. Recent studies suggest that almost half of the population suffers from mild to moderate periodontitis (Albandar 2011; Eke et al. 2012). Nevertheless, severe periodontitis is a disease occurring only in a minority of populations (8–15 %) (Albandar 2011; Eke et al. 2015).

Periodontitis is considered to be a complex disease: multiple factors play a role simultaneously in the pathophysiology (Loos et al. 2015). In periodontitis, the pathobiology behaves in a nonlinear fashion, where the disease progression rate fluctuates. The disease swings between periods of exacerbation to periods of quiescence (a disease-resolving state). In the active phase of the disease, the total of the host-related and lifestyle-related causal factors results in an aberrant immune reaction against bacteria in the gingival sulcus or pocket, in particular against gram-negative bacteria (Loos et al. 2015). The ensuing inflammatory reactions result in the described inflammatory infiltrate, proliferation, and ulceration of the pocket epithelium at the cost of sound tooth-supporting structures.

3.2.2 Atherosclerosis and Acute Atherosclerotic Events

Atherosclerosis is a disease of the whole vascular system, with obvious predilection places for more severe disease development (Friedewald et al. 2009; Andersen and Jess 2014; Garcia-Gomez et al. 2014; Pearson et al. 2003). Atherosclerosis is the gradual stiffening of arteries, the increasing thickness of the intima media

of vessels, and at special areas severe atherosclerotic plaque formation. This results in the narrowing of the arterial lumen. This is typically observed in the coronary, carotid, intercranial, and femoral arteries. Severe atherosclerosis and severe endothelial dysfunction can result in blockage and local thrombus formation in the artery preventing oxygen-rich blood to supply the distal tissues. This is regarded as an "ischemic event," such as an acute myocardial infarct, or stroke, or transient ischemic event in the brain.

The consequences of atherosclerosis are therefore the following:

1. Coronary artery disease (CAD) (also known as coronary heart disease (CHD)). This is a diagnosed, severe atherosclerotic plaque formation and narrowing of the lumen occurring in the coronary arteries, possibly resulting in angina pectoris and/or an acute myocardial infarction (MI) or acute cardiac death.
2. Cerebrovascular disease. This is confirmed severe atherosclerosis in carotid arteries and/or in intracranial vessels. The patient can experience transient ischemic accidents (TIA) mostly with reversible brain damage, or can suffer from a cerebrovascular ischemic accident (nonhemorrhagic stroke) with irreversible brain damage or sudden death.
3. Peripheral arterial disease (PAD). Severe atherosclerotic disease can also occur in peripheral arteries, such as the femoral arteries and/or tibial arteries, but also in the brachial artery. The patient can experience (transient) leg muscle pain (intermittent claudication); necrosis and open ulcers can develop and possibly thrombus formation, with the potential that the thrombus gets dislodged to lung tissue (pulmonary embolism).

The above cardiovascular conditions have been linked to periodontitis in many epidemiological studies, in both cross-sectional studies and in longitudinal studies. The latter type of studies has been prospective and retrospective. The review by Dietrich (2013) (Dietrich et al. 2013), presented and reviewed by the joint EFP/AAP workshop (Tonetti et al. 2013), concluded that there is evidence for the association of periodontitis with coronary artery disease, with cerebrovascular disease, mortality due to the latter conditions, and links with peripheral artery disease. However, to date, no firm evidence for any biological mechanism linking periodontitis to atherosclerosis and its sequelae is available. To what extent is periodontitis a true risk factor for atherosclerosis and how can it play a causative role?

We now further summarize current knowledge on the possible mechanisms on how periodontitis and atherosclerotic diseases may be linked (Schenkein and Loos 2013). In brief, the explanations for the associations focus on atherogenesis, that is, the development and exacerbation of atherosclerotic plaques (see Box 3.1); these include: (1) bacteremia, (2) a pro-inflammatory state, (3) a prothrombotic state, (4) an overactive immunity, (5) dyslipidemia, and (6) common genetic risk factors. Most likely, these six potential mechanisms do not act individually, but may play all simultaneously and may act in concert, making a patient with periodontitis more susceptible for atherogenesis in time, and more prone to suffer from an acute atherosclerotic event.

Box 3.1. Atherogenesis and the Atherosclerotic Lesion (Atheroma)

At some point, an initial atherosclerotic lesion starts (atherogenesis, atheroma formation). This results in activated and dysfunctional endothelial lining (1), and subsequently, partial loss of integrity of the lining of the blood vessel. Now, on and by these endothelial cells, there is an upregulation of adhesion molecules (ICAM-1, VCAM-1, E-selectin, P-selectin) and chemoattractants (e.g., IL-8, thrombin). These activated endothelial cells provide the adhesion triggers and receptors, for increased platelet and leukocyte adhesion to them. This results in diapedesis of monocytes and dendritic cells (both possibly with ingested bacteria), and also T cells, into the underlying inflammatory lesion.

Activated platelets may aggregate and may form mini-thrombi.

The inflammatory lesion (2) in the intima media of the artery may be in part initiated and/or propagated by bacteria originating from the periodontitis lesion. However, many more sources of bacterial remnants in such atherosclerotic lesions are conceivable and extensive bacterial signatures have been described (Ott et al. 2006). The inflammatory reactions in the atherosclerotic lesion can also be propagated by pro-inflammatory mediators (IL-1, IL-6, CRP, TNF-alpha) and chemotactic factors (e.g., MCP-1) both "spilled over" from the periodontal lesions and produced in the liver (8) and also systemically.

The atherosclerotic lesion increases over a period of years. Now it is characterized by the accumulation of degenerative material in the tunica intima (inner layer) of artery walls (3). The material consists of (mostly) a cellular infiltrate of macrophages and the latter fused to foam cells and lymphocytes ($CD4^+$ Th cells characterized by increased IL-12, IL-18, IFN-gamma production), further lipid streaks (cholesterol and fatty acids), calcifications, and a variable amount of fibrous connective tissue. The atherosclerotic plaque intrudes into the lumen of the artery and causes the arterial lumen to narrow, which will restrict blood flow. During atheroma maturation, one sees more and more modified low-density lipoproteins (LDL) phagocytosed within the macrophages and foam cells, resulting in an increase of pro-inflammatory cytokines (IL-6, IL-1, TNF-alpha), chemoattractants (IL-8), and metalloproteineases (MMPs). The active atherosclerotic lesion, now in a more inflammatory state, induces a further dysfunctional endothelial lining.

In the mature atheroma, a degeneration of smooth muscle cells and an increase of fibroblasts are observed (4), with progressive fibrosis and loss of a demarcation between the inflammatory lesion and smooth muscle cells; there is development of a compensatory blood supply for the affected artery and an increased outer muscle layer. In Fig. 3.1., items 2+3+4 form the intimal layer (media) of the artery, and item 5 is the outer muscle layer.

Ultimately, a disintegrated endothelial lining (6) can rupture, with exposition of the underlying atherosclerotic plaque. This generates thrombin from prothrombin, which in turn enzymatically generates fibrin from fibrinogen, initiating the clotting cascade and resulting in thrombosis and subsequently to a myocardial infarction, ischemic stroke, acute peripheral artery disease, or cardiac death.

This slow and chronic process over years of atherogenesis may be mediated by repeated bacteremia, a pro-inflammatory state and activated inflammatory cells, from infectious and inflammatory processes elsewhere (Andersen and Jess 2014; Koenig 2013). In this respect, Crohn's disease and ulcerative colitis, chronic periodontitis, but also rheumatoid arthritis have been implicated (Andersen and Jess 2014; Garcia-Gomez et al. 2014; Kholy et al. 2015).

Bacteria (transmigrated from the subgingival biofilm into the periodontal lesion or in the circulation itself) can activate platelets (7; out of scale within Fig. 3.1) and can trigger platelet–leukocyte complexes. These can form aggregates on the dysfunctional endothelium, in particular due to the adhesion molecules that are abundantly expressed, resulting in the initiation of (micro) thrombus formation.

Simultaneously, the liver (8) is stimulated and upregulated due to inflammatory signals (IL-6), to overproduce clotting factors, resulting in a prothrombotic state. Also increased production of acute phase reactants, including CRP, which

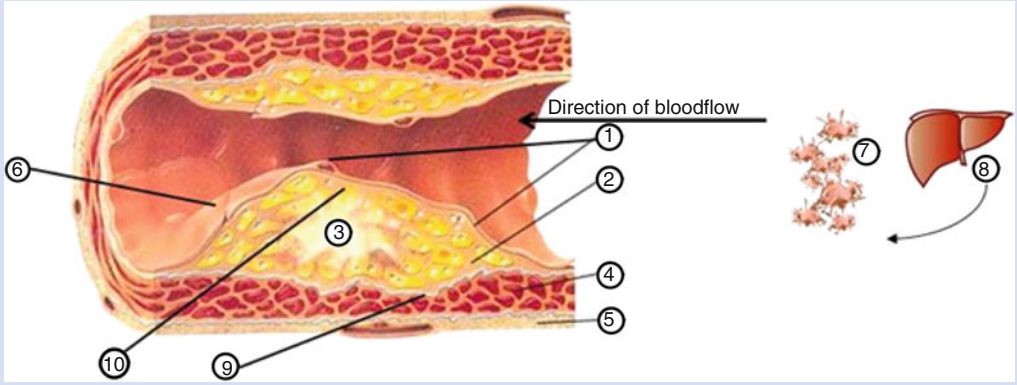

Fig. 3.1 Schematic cartoon of an atherosclerotic lesion and events in the process of atherogenesis (Adapted and reprinted from (Schenkein and Loos 2013))

will increase the pro-inflammatory state, are produced in the liver (8; again out of scale within Fig. 3.1).

Within the progressive atherosclerotic lesion, decreased collagen production and activation of MMPs (9) is occurring, resulting in reduction of the smooth muscle cell content and increased degradation of remaining collagen that borders the fibrous cap, weakening the strength of the vessel, leading to fissuring of the atheroma.

In a severely progressed stage (10), the atheroma comprises of a large necrotic core which is exposed to the vasculature within the lesion, leading to contact with platelets and initiation of coagulation and plaque rupture in so-called vulnerable lesions.

3.3 Bacteremia and Consequences for the Cardiovascular System

It has been known for many years that oral bacteria can enter the bloodstream. In particular, in cases of severe gingivitis and periodontitis, the inner epithelial lining of the periodontal pockets is ulcerated and shows loss of epithelial lining integrity (Schenkein and Loos 2013; Loos 2005; Tonetti and Graziani 2014). This ulcerated pocket epithelium may add up to 8–20 cm^2 (Loos 2005; Nesse et al. 2008). It is called periodontal inflamed surface area and forms an easy *porte d'entrée* for the bacteria, bacterial toxins, and other antigenic components residing in the subgingival area (Loos 2005; Nesse et al. 2008). Many of the subgingival bacteria are gram negative, and in particular, the endotoxin lipopolysaccharide (LPS) from these species stimulates the host immune system. This latter biologic event can be at the basis of the pro-inflammatory state seen in both periodontitis and cardiovascular disease (see below) (Schenkein and Loos 2013; Tonetti and Graziani 2014). Also the daily episodes of dissemination of bacteria from the periodontal lesions into the bloodstream can favor atheroma formation and can induce activated platelets and platelet–leukocyte complexes, leading to the prothrombotic state (see below).

It is now well documented that bacteria and endotoxins from periodontal lesions enter the bloodstream (Schenkein and Loos 2013; Tonetti

Table 3.1 Lines of evidence for the role of bacteria and specifically periodontal pathogens and atherosclerosis (Tonetti and Graziani 2014)

Evidence for the role of periodontal pathogens in atherosclerosis
DNA of periodontopathogens has been localized within atherosclerotic plaques
Periopathogens are able to invade endothelial cells, in particular *P. gingivalis*
Periopathogens and other oral bacteria may trigger platelet aggregation
P. gingivalis can accelerate the transition of macrophages to foam cells
Periopathogens can activate the host immune system and cause a pro-inflammatory state

and Graziani 2014; Reyes et al. 2013). This has been shown when patients chew, brush their teeth, but also after dental procedures. However, the long-standing periodontal lesions and the chronic daily short-lived bacteremias are mostly responsible for the negative effects. The possibilities for oral and periodontal bacteria to cause harm for the cardiovascular system and to be involved in atherogenesis and atherosclerosis are summarized in Table 3.1.

Interestingly, some indirect evidence for a role of bacteria in atherogenesis or in atherosclerotic lesions in general is the finding of a large variety of bacterial signatures in atherosclerotic biopsies (Ott et al. 2006). Specifically, in addition to a high variety of species, also *P. gingivalis*, *A. actinomycetemcomitans*, and other periodontal pathogens have been identified (Table 3.1). In periodontitis, the prevalence of bacteremias and endotoxinemias is higher than in gingivitis or periodontal healthy subjects as evidenced by higher microbial diversities in atherosclerotic biopsies from periodontal patients. Also, there are correlations between the presence of bacterial antigens and molecular signatures in the atherothrombotic lesions and the severity of periodontitis. Moreover, there are correlations reported between the composition of the subgingival microflora and the bacterial species in vascular biopsies. Even, interestingly, there is suggestion that some bacterial species are still viable.

Taken together, there is biological evidence that bacteria and their toxic components, such as LPS, may easily gain access to the vascular system in cases of periodontitis. And biological experiments have demonstrated that this phenomenon plays a role in atherogenesis and exacerbation of atherosclerotic lesions.

3.4 Immunologic Reactions

The increased bacteremias and dissemination of endotoxins (LPS) and many other microbiological antigens stimulate the immune response of the host (Schenkein and Loos 2013). Consequently, an activated innate immunity may result in hyperactive neutrophils, higher levels of complement, increased levels of acute phase reactants (see below), and a general exacerbation of reactivity.

Also the adaptive immunity is activated. The latter is an intriguing finding and the consequences are not well understood. It is found that there is an increased titer of antibodies to oral and periodontal bacteria in the circulation in patients with periodontitis. These antibodies first of all can enter atherosclerotic lesions where such oral species are nested. This can exacerbate such atherosclerotic lesions and could increase the endothelial dysfunction and increase the risk of rupture of the lesion with thrombus formation as a result.

Furthermore, enhanced T- and B-cell activation may give rise to autoimmunity. Autoantibodies may be generated through the molecular mimicry between bacterial and human heat shock proteins (HSP). These cross-reactive antibodies in the first place generated against circulating microorganisms may react, for example, with HSPs on endothelial cells. These become dysfunctional and again this can enhance the chance for rupture of the endothelial lining giving rise to thrombus formation with the chance of ischemia. Anti-HSP antibodies against the periodontal pathogens *P. gingivalis, F. nucleatum, T. forsythia*, and *A. actinomycetemcomitans* have been found. Also the activated T and B cells against other antigens of these bacteria have been found. Further, other autoantibodies include anticardiolipin and anti-low-density lipoproteins (anti-LDL-type cholesterol) (Schenkein and Loos 2013; Schenkein et al. 2004).

3.5 Pro-inflammatory State

It has been widely accepted that low-grade systemic inflammation contributes to an elevated risk for CAD and ischemic stroke (Friedewald et al. 2009; Andersen and Jess 2014; Garcia-Gomez et al. 2014; Pearson et al. 2003; Koenig 2013; Emerging Risk Factors Collaboration et al. 2010; Ridker 2009). In the last two decades, C-reactive protein (CRP) has been proposed as an important inflammatory biomarker related to acute atherosclerotic events. CRP levels exhibit a continuous association with the risk of CAD, ischemic stroke, and vascular mortality (Emerging Risk Factors Collaboration et al. 2010). Interestingly, lowering of CRP levels after statin therapy is associated with a decrease in cardiovascular disease event rate, equivalent to what was observed for patients who achieved lowered LDL cholesterol treatment goals (Ridker et al. 2005).

CRP is an "acute-phase reactant," a molecule produced mainly in the liver, in response to inflammatory signals, in particular in reaction to elevated levels of interleukin-6 (IL-6). The acute-phase reactants such as CRP have pro-inflammatory properties. The exact function(s) of CRP, despite its years of discovery as biomarker, is not yet completely uncovered. CRP is a pentatrexin molecule; it functions as soluble pattern recognition molecule, and one of the most important roles for this protein is host defense primarily against pathogenic bacteria. It can function as an opsonin for pathogens through activation of the complement pathway and through binding of Fc-gamma receptors. CRP and other pentatrexins can also recognize membrane phospholipids and nuclear components exposed on or released by damaged endothelial cells. In this way, it is proposed to be important for clearance of damaged cells and tissues. Because CRP is a strong acute-phase reactant, it is widely used as a diagnostic marker for acute inflammation and/or infection, with levels ranging 10–200 mg/l or even higher.

The levels of CRP related to chronic inflammation and proposed as cardiovascular risk biomarker are relatively low and not exceeding 10 mg/L. These levels have been found by applying a high sensitivity test using nephelometry and therefore often referred to as hsCRP. Levels of hsCRP are classified in three categories with regard to risk for cardiovascular events (Table 3.2) (Pearson et al. 2003).

Table 3.2 Risk levels for acute cardiovascular events based on plasma hsCRP levels

Risk for acute cardiovascular events	Levels of hsCRP
Low	<1 mg/L
Intermediate	1–3 mg/L
High	>3 mg/L

In periodontitis, consistently elevated levels of hsCRP have been found (Paraskevas et al. 2008). Meta-analysis of published case-control studies have shown that in periodontitis often CRP levels are in the range of intermediate to high CVD risk, while the values in controls are in the low- or intermediate-risk category. The latter authors reported that the weighted mean difference between patients and controls was highly significant (1.56 mg/L). Moreover, it has been shown that hsCRP behaves in a dose-dependent manner: severe periodontitis in general shows higher blood plasma levels of hsCRP than moderate or mild periodontitis, while healthy controls show even lower values.

Furthermore, a meta-analysis on periodontitis treatment studies ($n = 23$) including 1647 patients have shown that hsCRP levels are significantly reduced after periodontal therapy (overall a mean reduction of 0.5 mg/L) (Teeuw et al. 2014). The reduction was specifically significant in a subgroup of periodontitis patients having comorbidities such as diabetes, atherosclerosis, or rheumatoid arthritis. The reduction of hsCRP in periodontitis treatment studies is clinically relevant since the hsCRP levels exhibit a continuous association with the risk of CAD, ischemic stroke, and vascular mortality (Emerging Risk Factors Collaboration et al. 2010); the hsCRP levels after periodontal treatment can often place patients in a low CVD risk category (Table 3.2). These treatment studies with favorable results suggest further that periodontitis is causally related to elevated CRP.

Next to hsCRP are other markers of inflammation in the circulation of patients with periodontitis.

Also these can make periodontitis patients more susceptible for an atherosclerotic event as they have atherogenic potential (Schenkein and Loos 2013). These include other acute-phase reactants and immune mediators: interleukin (IL)-1, IL-4, IL-6, IL-18, haptoglobin, serum amyloid A, alpha 1 anti-chymotrypsin, tumor necrosis factor-alpha, metalloproteinase (MMP)-9, platelet-activating factor (PAF), and PAF acetylhydrolase.

The pro-inflammatory state as characterized by the elevated levels of hsCRP, and other biomarkers mentioned above, could enter the blood circulation in essentially two ways:

1. The spill over from periodontal lesions: There is substantial literature providing indications that inflammatory cytokines and other biomarkers are produced in the periodontal lesion; from here they are "dumped" or "spilled" into the blood circulation. These molecules could impact organs and tissues, such as blood vessels with/without atherosclerotic lesions and the liver.
2. The activated liver: The liver in turn initiates an acute-phase response. The liver produces higher levels of CRP, and other acute-phase reactants, but also higher levels of complement molecules and prothrombotic molecules, such as fibrinogen, von Willebrand factor, and plasminogen.

In summary, through the clearly proven pro-inflammatory state in periodontitis, there is a highly plausible biologic mechanism why periodontitis is linked to atherosclerosis and acute atherosclerotic events. Excessive local production of pro-inflammatory cytokines, as well as higher levels of these *and* acute-phase reactants produced in the liver, can induce or exacerbate inflammatory changes in the endothelium and atherosclerotic lesions (Fig. 3.1).

3.6 Prothrombotic State

In recent years, there is a growing body of evidence that in periodontitis both a hypercoagulable state and hypofibrinolysis exist (Schenkein and Loos 2013; Tonetti and Graziani 2014). Collectively, we call this a prothrombotic state, i.e., an individual may form faster than normal a (small) thrombus *and/or* this is less efficiently removed. It is hypothesized that this state in periodontitis could also be one of the mechanisms as to how periodontitis is associated with ischemic atherosclerotic events. In Box 3.2, we summarize the normal blood clotting events.

Box 3.2. The Normal Blood Clotting Events

In response to vascular injury, a sequence of coordinated events ensures blood fluidity while preventing blood loss. Adhesion, activation, and aggregation of platelets are all steps in primary hemostasis. Under static or low shear conditions of flow, platelets circulate in the inactivated discocyte form. Upon vascular or tissue injury, collagen matrix exposure and local adenosine diphosphate (ADP) production result in agonist–receptor interactions (GPIb-von Willebrand factor, GPVI-collagen, P2Y12-ADP), which conclude with formation of platelet aggregates.

Formation of the platelet hemostatic plug is followed by true blood clot formation (activation of coagulation) and, finally, by clot dissolution (fibrinolysis). Activation of coagulation is strongly dependent on upregulation of tissue factor (TF) and leads to thrombin generation. TF is not normally present in the circulation. Normal hemostasis arises when the blood vessels are disrupted allowing blood to contact extravascular cells expressing TF. Thrombin is active in the conversion of soluble fibrinogen to insoluble fibrin (deposited in the formed blood clot) and is a potent platelet activator. There are three natural anticoagulant mechanisms acting together to prevent thrombosis: (I) the heparin–antithrombin system, (II) the protein C pathway, and (III) the tissue factor pathway inhibitor system.

Fibrinolysis is a natural response to coagulation, leading to fibrin clot breakdown.

During fibrinolysis, plasmin is generated from plasminogen (an inactive proenzyme) under the influence of tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA); this reaction is inhibited by plasminogen activator inhibitor 1 and 2 (PAI-1, PAI-2) and α2-antiplasmin. The formed plasmin cleaves fibrin into soluble degradation products.

Table 3.3 Changes or abnormalities in hemostasis parameters observed in periodontitis

Hemostasis parameters	Situation in periodontitis
Biomarkers of coagulation	
Fibrinogen	↑
Fragment 1 + 2	↑
von Willebrand factor	↑
P-selectin	↑
Abnormalities of platelets	
Numbers in the circulation	↑
Size (mean platelet volume)	↑
Activation state	↑
Reactivity	↑
Abnormalities of fibrinolysis	
tPA	↓
PAI-1	↑
D-dimer	↑

Abbreviations: *tPA* tissue-type plasminogen activator, *PAI-1* plasminogen activator inhibitor 1

In periodontitis, the normal hemostasis state and events may be disturbed. In Table 3.3, we summarized the findings. One of the very first parameters found to be elevated in periodontitis was fibrinogen (Kweider et al. 1993). Fibrinogen is a member of the acute-phase protein family. Its concentration rises in acute and chronic inflammation mainly as result of increased production by hepatocytes. Increased concentrations of fibrinogen are associated with the development of atherothrombotic disease. Several cohort and population-based studies have documented an increased level of plasma fibrinogen in periodontitis patients compared to periodontally healthy individuals (Kweider et al. 1993; Papapanagiotou et al. 2009; Wu et al. 2000). Studies on the effects of periodontal therapy on fibrinogen levels (reviewed in (Teeuw et al. 2014; D'Aiuto et al. 2013)) yielded variable results; some studies reported a significant reduction in the fibrinogen levels in the patient groups that received active periodontal treatment; others measured no significant changes post-therapy.

Other markers of coagulation or fibrinolysis (prothrombin fragments 1 + 2, D-dimer, von Willebrand factor, tissue plasminogen activator and plasminogen activator inhibitor-1) known to be modified in prothrombotic states, rendered inconclusive results when explored in periodontitis. Bizzarro et al. (Bizzarro et al. 2007) reported increased levels of PAI-1 (Bizzarro et al. 2007) and the levels of PAI-1 decreased after full-mouth extractions (Taylor et al. 2006), whereas another study failed to demonstrate an association between PAI-1 and periodontitis (Bretz et al. 2005). Interestingly, there is evidence for a short-term increase in the first week after periodontal treatment of hemostatic factors such as PAI-1, D-dimer, and von Willebrand factor (D'Aiuto et al. 2005a, b; Tonetti et al. 2007; Graziani et al. 2010). These changes correspond to an acute-phase reaction in response to the treatment itself measurable by an increased CRP, IL-6, possibly related to the acute bacteremia following full-mouth subgingival debridement.

In response to hemorrhage, circulating platelets adhere to exposed subendothelial tissues and recruit additional platelets into aggregates that function as procoagulant surfaces. Platelets are released in the bone marrow from precursors, the megakaryocytes. The process is controlled by thrombopoietin, a liver-synthesized hormone, which stimulates multipotent megakaryoblasts toward maturation. In patients with reactive high platelet counts secondary to inflammation, pro-inflammatory cytokines such as IL-6 are responsible for enhanced hepatic production of thrombopoietin, resulting in increased platelet production. Untreated periodontitis has been associated with elevated numbers of platelets (Papapanagiotou et al. 2009; Lopez et al. 2012; Wang et al. 2014), and the association of high platelet counts in periodontitis is further

strengthened by the observations that platelet numbers decrease after periodontal therapy (Wang et al. 2014; Christan et al. 2002).

Platelets play a crucial role in the pathogenesis of atherosclerotic complications, contributing to thrombus formation or apposition after plaque rupture. The mean platelet volume (MPV) is universally available with routine blood counts and is a quantity of the average size of platelets in a sample. Compared to smaller ones, larger platelets are more reactive, have more granules, aggregate more rapidly with collagen, have higher thromboxane A2 level, and express more glycoprotein Ib and IIb/IIIa receptors. Elevated MPV levels have been identified as an independent risk factor for myocardial infarction in patients with coronary heart disease and for death or recurrent vascular events after myocardial infarction. In chronic periodontitis, there is an ongoing low-grade inflammation, and cytokines such as IL-6 or IL-3 regulate megakaryocyte ploidy, resulting in the production of more reactive and larger platelets. Therefore, we can expect higher MPV values in untreated periodontitis. Indeed, higher MPV in periodontitis patients than in healthy controls have been reported (Lopez et al. 2012). In contrast, Wang and coworkers found a lower MPV in periodontitis patients at intake, and the MPV increased without reaching the levels of healthy controls 1 month post-periodontal therapy (Wang et al. 2014, 2015). The source of these conflicting results is as of yet unknown, but there are some plausible explanations. Lower MPV in untreated periodontitis might be the effect of intensive consumption of large platelets at sites of overt inflammation. Post-therapy, larger platelets may represent newly released, young platelets during rebound from platelet clearance. However, MPV variations should be interpreted with caution in unmatched cohort studies, as confounding factors, such as body mass index, systolic and diastolic blood pressure, smoking status, glucose and cholesterol levels, or medication use have all been associated with MPV variations.

As described above, regularly occurring bacteremias in periodontitis patients underlie chronic production and systemic increases of various pro-inflammatory immune mediators. These could be the cause for the observed platelet activation and reactivity (Table 3.3). Interestingly, strains of the recognized periodontal pathogens *A. actinomycetemcomitans* and *P. gingivalis*, but also other dental plaque bacteria, such as *Streptococcus sanguis*, induce platelet activation and aggregation in vitro and in animal studies (Nicu et al. 2009; Assinger et al. 2011, 2012). Platelets become activated by periodontopathogens mainly via toll-like receptor 2 (TLR2) and TLR4.

P-selectin is a transmembrane protein present in the Weibel–Palade bodies of endothelial cells and in the α-granules of platelets. P-selection is expressed on the cell surface upon activation-dependent granule exocytosis and plays a central role in cardiovascular disease. Upon interaction with its receptor P-selectin glycoprotein ligand 1 (PSGL-1) on the leukocyte surface, P-selectin (both platelet, as well as endothelial cell derived) is rapidly shed to form soluble P-selectin. Elevated levels of plasma P-selectin have been documented in patients with periodontitis (Papapanagiotou et al. 2009; Assinger et al. 2011).

Another cytokine expressed and released from activated platelets is the CD40L (cluster of differentiation 40 ligand); in fact, platelets represent the main source of soluble CD40L. Ligation of CD40L to its receptor CD40 induces a pro-inflammatory and prothrombotic response in the vascular endothelium, as evidenced by the release of inflammatory cytokines, expression of adhesion molecules, activation of matrix metalloproteinases (MMPs), and procoagulant tissue factor. It initiates the formation of reactive oxygen species and inhibition of nitric oxide production. Also elevated levels of soluble CD40L were found in periodontitis patients, and these were correlated with P-selectin (Papapanagiotou et al. 2009; Assinger et al. 2012), highly suggestive of an activated platelet phenotype in periodontitis. The platelets were not only activated, but also hyperreactive. In response to several species of oral bacteria, platelets from periodontitis patients showed an increased membrane exposure of P-selectin and increased formation of platelet–monocyte complexes compared with controls (Nicu et al. 2009). Formation of platelet–leukocyte complexes is a process that facilitates leukocyte transmigration to

perivascular tissues. However, these interactions also occur in circulating blood, leading to activated platelet–leukocyte aggregates, which are hallmarks not only of inflammatory disorders and sepsis but also of acute myocardial infarction.

It is clear that in periodontitis small aberrations from normal can be found regarding the hemostasis physiology. Most is based on cross-sectional case-control studies or longitudinal cohort follow-up studies. Since periodontitis is treatable, future longitudinal research should address the question of whether periodontal therapy is capable of reducing the levels of hemostasis biomarkers and reducing the platelet activation and reactive state. These studies will also help to further confirm a mechanistic role of the pro-inflammatory state in periodontitis being causally related to acute ischemic events.

3.7 Dyslipidemia

For more than four decades, the role of high cholesterol in atherosclerosis and the acute atherosclerotic events has been established. Total cholesterol consists mainly of HDL (high-density lipids) and LDL (low-density lipids). Increased serum levels of especially LDL and very low-density lipoproteins (v)LDL and triglycerides are considered pro-atherogenic. The (v)LDL can actually diffuse freely into the intimal layer of blood vessels. In atherosclerotic lesions, one finds foam cells, being multinuclear macrophages which have phagocytized LDL. Regular macrophages, not yet fused into multinuclear cells, are activated when they have phagocytized LDL and vLDL and may exuberate inflammation in the atherosclerotic lesion. Subsequently, the endothelial cells overlaying the atherosclerotic lesion become dysfunctional and express many chemokines and surface receptors.

Actually not only diet and "fatty foods," but in general, inflammatory processes seem to be associated with dyslipidemia, i.e., increased levels of (v) LDL and decreased levels of HDL. In fact, cholesterol is synthesized in the liver; it is not only acquired via diet. When the liver is activated by cytokines directly via pro-inflammatory cytokines, higher levels of cholesterol are generated. This is called elevated biosynthesis of cholesterol in the liver. Case-control studies have noted elevated total serum cholesterol, elevated LDL and lowered HDL, and elevated (v)LDL and intermediate-density lipoproteins in periodontitis (Schenkein and Loos 2013). Also increased triglycerides (TGs) have been found in periodontitis.

LDL cholesterol can bind to circulating LPS. The LDL–LPS complex in particular is very atherogenic. The LDP–LPS complex can enter easily atherosclerotic plaques and enhance the inflammatory reactions/responses inside the atheromas. The latter was also observed in in vitro experiments.

Moreover, LDL can be oxidized (oxLDL) and autoantibodies to oxLDL have been observed in periodontitis (Schenkein et al. 2004). Interestingly, *P. gingivalis* can induce foam cell formation in the presence of exogenous LDL. In sum, from cross-sectional clinical studies, we see that periodontitis is associated with dyslipidemia, i.e., elevated total cholesterol and elevated LDL and TGs, with concomitant lower levels of HDL. This total makes periodontitis patients with dyslipidemia more susceptible for increased atherogenesis, in particular in connection with LPS.

A recent review and meta-analysis (Teeuw et al. 2014) has demonstrated that dyslipidemia in periodontitis patients can be reduced following periodontal therapy. In the context of the current discussion on possible mechanisms how periodontitis might be linked to atherosclerosis, this is another clear indication that periodontitis is actually related to dyslipidemia. But also it is important in terms of prevention of further development of atherosclerotic lesions; treatment of periodontitis can be helpful, or can be part of a set of treatments, to lower an individual's risk for an acute atherosclerotic event.

3.8 A Common Genetic Background

Recent papers have identified the same genetic variants (single nucleotide polymorphisms [SNP]) to be associated with both coronary artery

disease (CAD) and periodontitis. This is a very intriguing finding. A common genetic background for CAD and periodontitis could be interpreted that the host acts in similar – aberrant – ways to infections and/or inflammatory processes, irrespectively where they take place. As stated before, atherosclerotic plaques are essentially inflammatory lesions. For example, we could think about similar host immune reactions and similar pathobiologic pathways to bacteria and bacterial antigens that are transmigrated or dislodged or phagocytized in macrophages/foam cells in atherosclerotic plaques, as well as host reactions to the bacteria and bacterial components which are entered into periodontal lesions from the subgingival biofilm.

One of the first and best replicated genetic loci for CAD is the *ANRIL* locus. The *ANRIL* locus is a regulatory region and does not contain a protein-encoding gene. It is a long noncoding antisense RNA formerly called CDKN2BAS. Importantly, it appears to be a pleiotropic genetic region, since it is also associated with diabetes type 2, ischemic stroke, and Alzheimer disease. Since 2009, it is reported that certain genetic variations in *ANRIL* are also consistently associated with periodontitis (Schaefer et al. 2009; Ernst et al. 2010). Further, a conserved noncoding element within *CAMTA1* upstream of *VAMP3*, also first identified as a genetic susceptibility locus for CAD, was found to be associated with periodontitis (Bochenek et al. 2013). Experimental work suggests that ANRIL and VAMP3 are part of biological pathways (regulatory networks) that connect glucose and fatty acid metabolism steps with immune responses (Bochenek et al. 2013; Schwenk et al. 2012). Interestingly, a genome-wide association study suggested that *VAMP3* locus was associated with a higher probability of subgingival colonization of periodontal pathogens (Divaris et al. 2012). Collectively, although speculative, these shared genetic factors suggest a mechanistic link between CAD, periodontitis, diabetes, metabolic syndrome, obesity, and inflammation. The impairment of the regulatory pathways by genetic factors may be a common pathogenic denominator of at least CAD and periodontitis (Schaefer et al. 2015; Loos 2015). We hypothesize that an aberrant inflammatory reactivity, determined in part by genetic variants in ANRIL, CAMTA1 and VAMP3, could also explain the epidemiological link between periodontitis and atherosclerosis in coronary arteries (CAD).

And recently, yet another CAD risk locus was also found to be associated with periodontitis; there is now evidence for *PLASMINOGEN (PLG)* as a shared genetic risk factor of CAD and periodontitis (Schaefer et al. 2015). When plasminogen is converted into plasmin, the latter enzyme can dissolve the fibrinogen fibers that entangle the blood cells in a blood clot; this is called fibrinolysis. Thus, the plasminogen–plasmin axis has an important function in tissue degradation and control in the blood coagulation system. Interestingly, bacteria (including *P. gingivalis*) can aggregate with plasminogen and can convert plasminogen to plasmin, and this complex is highly proteolytic and can inactivate plasmin inhibitors causing perhaps uncontrolled plasmin activity. Although we have no clear picture yet on the precise consequences of genetic variants in *PLG*, more and more pleiotropic genetic regions are identified and may form the basis for common diseases such atherosclerosis and periodontitis (Vaithilingam et al. 2014); then, periodontitis is not causally related to atherosclerosis, but rather the sequel of common aberrant inflammatory pathways.

3.9 Perspective and Concluding Remarks

Epidemiological studies have clearly indicated beyond any doubt that atherosclerosis and atherosclerotic events (CAD, cerebrovascular disease, PAD, and death due to ischemic events) are associated with periodontitis. However, it is important to note that it is not proven that periodontitis plays a causative role in the pathophysiology of atherogenesis or atherosclerosis. Atherosclerosis, actually the presence of atherosclerotic lesions that slowly progress and can rupture with acute MI or stroke as consequence, is considered a complex disease. Like for periodontitis, complexity of atherosclerosis means that it is a disease process involving multiple causal components,

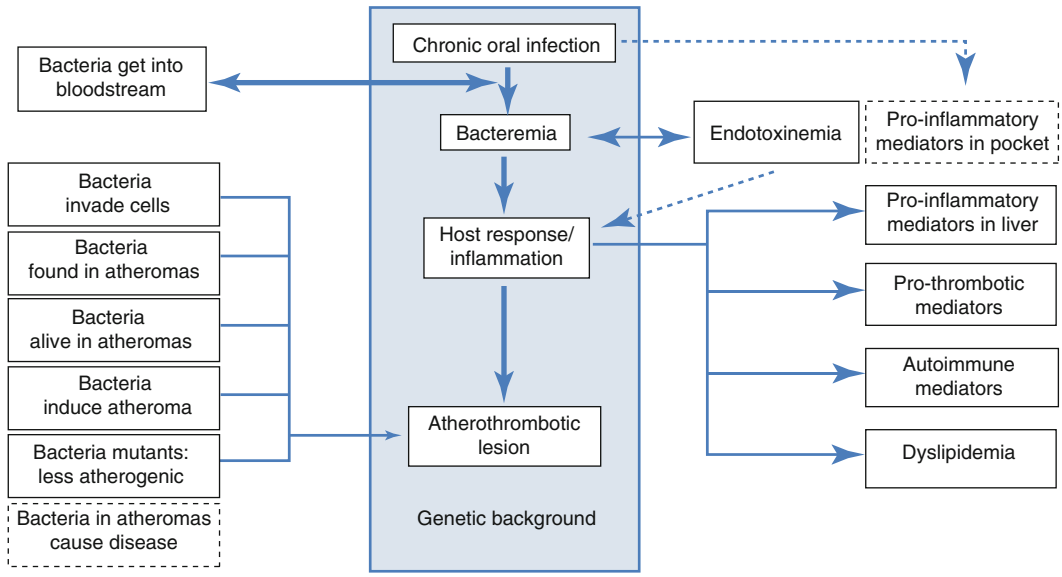

Fig. 3.2 A composite of biologically plausible mechanisms how periodontitis might be linked to atherogenesis and atherosclerosis (Reprinted with permission for Tonetti et al. (2013) and based on references (Schenkein and Loos 2013; Reyes et al. 2013))

which interplay with each other simultaneously. In complex systems, the causes and effects may behave disproportional, so that a small cause may result in a large effect, and vice versa, and that the disease progression rate fluctuates or, rather, can move from a non-acute or chronic state to an acute phase without a special "warning signal." Complexity of atherogenesis and atherosclerosis reveals heterogeneity in its clinical course and in the various phenotypes between diseased patients.

There are several main causal risk factors for atherosclerosis to occur, including: (i) genetic risk factors and epigenetic modifications of the genetic code; (ii) lifestyle-related factors, such as diet and fat intake (high/low cholesterol-containing foods); (iii) systemic diseases such as diabetes and its sequelae on the condition of the blood vessel walls, smoking, high blood pressure, and obesity; (iv) now also chronic inflammatory processes and/or chronic infections are considered as another cause for atherosclerosis. It is important to understand that, while these factors mentioned above play simultaneously a role in the pathobiology of atherosclerosis, the relative contribution of each of the causal factors varies from patient to patient. For example, not every patient who suffered from an acute MI may have had high cholesterol or was a smoker.

In this chapter, we have outlined possible mechanisms, as to how periodontitis may be another risk factor for atherosclerosis. These have been discussed before in the EFP/AAP workshop (Tonetti et al. 2013) and are summarized in Fig. 3.2.

For the link of periodontitis with atherosclerosis, researchers suggest that in fact the daily occurrence of multiple short-lived bacterial disseminations may be at the base of a possible causal role of periodontitis. But also the fact that periodontitis is a chronic inflammatory disease, which can cause a spillover of pro-inflammatory cytokines and induces a pro-inflammatory state, dyslipidemia and a prothrombotic state, can present the risk to increased atherogenesis and/or enhanced pathology of atherosclerotic lesions. However, a common genetic susceptibility for atherosclerosis and periodontitis (and perhaps other linked chronic diseases) could dictate the way the host responds in general to certain types of inflammatory processes. These diseases may share the same inflammatory pathways in reaction

to, for example, bacteria, be it in the gingival tissues or be it in the circulation or atherosclerotic lesion. Some pleiotropic genes have been identified, such as *ANRIL*, *CAMTA1*, and *PLG*.

Independently, whether there is a true causal contribution of periodontitis to atherosclerosis, periodontitis treatment studies have indicated that the clinical condition of the vascular system can improve, i.e., the degree of atherosclerosis can be reduced by periodontal therapy (Tonetti et al. 2013; Teeuw et al. 2014). For example, endothelial dysfunction can be decreased after periodontal therapy and other studies showed a decrease in the intimedia thickness of the carotid arteries (Loos 2015). Also hsCRP is clearly reduced after periodontal therapy and even some studies showed a reduction in blood pressure and dyslipidemia. With these clinical findings in mind, the proposed mechanisms as to how periodontitis may be linked to atherosclerosis and its sequelae seem plausible. Obviously, much more fundamental and clinic research is needed to further study the associations between periodontitis and atherosclerotic diseases.

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Linkage Between Periodontal Disease and Diabetes Mellitus

4

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Abstract

The past decades have significantly widened the perspectives of the chronic oral infectious disease known as periodontitis. The disease is regarded as a bacterial infection resulting in low-grade inflammation of the periodontal tissues, and both the associated release of pro-inflammatory mediators and the presence of bacteria in the periodontal pockets, which, as the result of daily procedures, may spread after penetration of the vasculature, are possible mediators of systemic consequences. This chapter deals with the possible association between periodontitis and diabetes mellitus which is believed to possess in a two-way interrelationship.

4.1 Diabetes Mellitus

Diabetes mellitus (DM) comprises a heterogeneous group of disorders, characterized by increased blood glucose level (Bell and Polonsky 2001). The two most common forms are type 1 and type 2 DM. While type 1 DM is due to an autoimmune reaction of polygenic origin with destruction of the insulin-producing β cells of the pancreas, resulting in insulin deficiency, type 2 DM is related to insulin resistance at cellular and organ levels and altered lipid metabolism due to inactivity, high intake of food, and obesity in genetically susceptible individuals. As compensation, the β cells in type 2 DM patients are stimulated to increase their insulin secretion, but this compensatory mechanism may over time become insufficient to maintain the blood glucose level within the normal range, thereby resulting in overt type 2 DM (Kahn 2001; Nolan et al. 2011). Type 2 DM is the most widespread endocrine disorder and the prevalence of the disease is increasing due to a worldwide growth in number of people with overweight and obesity. Thus, in 2025–2030, it is estimated that well above 300 million individuals

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worldwide will suffer from type 2 DM, the prevalence being above 6 % (Zimmet et al. 2001; Wild et al. 2004; Kaul et al. 2012). Globally, type 1 DM, which is the predominant DM type among younger individuals, accounts for 5–10 % of total DM cases (American Diabetes Association 2009; SEARCH for Diabetes in Youth Study Group and Liese 2006). Type 2 DM, accounting for the remaining DM cases, was previously considered a disease of the elderly, but is increasingly seen in younger generations, now also in children and young adults (Pinhas-Hamiel and Zeitler 2005). Prediabetes is part of the natural history of type 2 DM and it is defined by American Diabetes Association (ADA) as "a condition, in which blood glucose levels are higher than normal, but not yet diabetic, known as impaired glucose tolerance or impaired fasting glucose" (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997). The condition is highly prevalent, and a significant number of patients with prediabetes develop type 2 DM within 10 years (Benjamin et al. 2003).

A significant proportion of up to 50 % of individuals currently suffering from type 2 DM presumably remains undiagnosed (Glumer et al. 2003, Guariguata et al. 2011), which result in persistent poor metabolic control in individuals with unrecognized type 2 DM. Poor metabolic control in DM patients is associated with a number of complications including low-grade inflammation and macrovascular and microvascular changes, including cardiovascular disease (CVD), eye and kidney problems, impaired wound healing, and increased prevalence and severity of periodontitis (Morain and Colen 1990; Löe 1993; Valensi et al. 1997; Stratton et al. 2000; King 2008). Among the macrovascular changes, accelerated atherosclerosis is part of the development of CVD and cerebrovascular events (Kannel and McGee 1979; Manson et al. 1991). The microvascular changes may result in renal failure and blindness (Anonymous 1996). DM has been known for years to be an important risk factor for periodontitis, and periodontitis is increasingly considered a late complication of DM (Löe 1993; Preshaw and Bissett 2013). An overall estimate for risk of periodontitis in DM patients is increased by 2–3 times (Casanova et al. 2014).

4.2 Association of Periodontitis and DM

4.2.1 Population Data

In the recent decades, the relationship between DM and periodontitis has been subjected to increasing scientific interest because the prevalence of type 2 DM is increasing, and because the bidirectional interaction between the two diseases has major impact on the affected patients. Dentists and medical doctors should be aware of the interaction between the two diseases.

A large body of cross-sectional and longitudinal studies have demonstrated that both type 1 and type 2 DM patients suffer more periodontitis than do nondiabetic patients (Glavind et al. 1968; Hugoson et al. 1989; Thorstensson and Hugoson 1993; Grossi et al. 1994, 1995; Dolan et al. 1997; Skrepcinski and Niendorff 2000; Xavier et al. 2009; Ochoa et al. 2012; Poplawska-Kita et al. 2014, for recent reviews, see Casanova et al. 2014; Wu et al. 2015). Gingival inflammation in type 1 diabetic children and adolescents is more common than in nondiabetic controls (Ryan et al. 2003). Further, gingivitis is more common in adults with type 2 DM than in nondiabetic controls (Orbak et al. 2008), and good metabolic control appears to reduce the prevalence of gingivitis (Albandar and Tinoco 2002). The progression from gingivitis to periodontitis is dependent on the level of metabolic control. Thus, poor metabolic control is an important determinant of periodontal breakdown in DM patients (Tervonen and Karjalainen 1997; Iughetti et al. 1999; Garcia et al. 2015), and since poor metabolic control is often associated with poor oral hygiene, it has been suggested that dental health education is particularly important in this population (Aggarwal and Panat 2012). A recent European study confirmed that well-controlled type 2 DM is not associated with periodontitis and neither does prediabetes associate with periodontitis (Kowall et al. 2015). Moreover, the prevalence of periodontal sites with moderate to severe attachment loss depends on the duration of type 2 DM (Al-Khabbaz 2014).

There is growing evidence that periodontitis may aggravate the course of DM, but the effect of periodontitis on glycemic control in type 1 DM patients is controversial. However, evidence shows a direct correlation between periodontal health and glycemic control in type 2 DM patients (Lakschevitz et al. 2011). A systematic review of epidemiologic observational studies concluded that periodontal disease adversely affects DM outcomes, i.e., metabolic control and development of late complications, but also that further longitudinal studies are warranted (Borgnakke et al. 2013). Thus, clinical investigations have associated periodontitis with increased risk of complications in DM patients (Saremi et al. 2005) and with increased HbA1c in nondiabetic patients (Demmer et al. 2010).

The best evidence of the significance of periodontitis for the course of DM probably comes from clinical studies on the effect of periodontal treatment in DM patients (see below).

4.2.2 Biological Similarities

Both DM and periodontitis may be associated with a state of low-grade inflammation. Thus, dysregulation of the cytokine production is essential for the pathogenesis of DM (Kolb and Mandrup-Poulsen 2010), and pro-inflammatory cytokines are increased in both diseases, including tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β), IL-6, and IL-18. These increased cytokine levels may contribute to insulin resistance and to diabetic complications, as well as to destruction of pancreatic β cells (Johnson et al. 2006; Graves and Kayal 2008; Nikolajczyk et al. 2011; Cruz et al. 2013). Likewise, pro-inflammatory cytokines produced locally in the inflamed periodontal tissues, where they are involved in tissue-destructive processes, may spill into the circulation with systemic impact and contribute to a state of low-grade inflammation (Amar et al. 2003; Elter et al. 2006; Garlet 2010). Interestingly, adipose tissue is an important source of cytokine production, and obesity may predispose to both type 2 DM and periodontitis (Hotamisligil et al. 1993; Genco et al. 2005; Pischon et al. 2007; Saito and Shimazaki 2007; Lontchi-Yimagou et al. 2013), although the significance of obesity for periodontal tissue degradation is still to be resolved (Kongstad et al. 2009). An obvious similarity of the two diseases is the increased level of oxidative stress (Bullon et al. 2009).

The role of antibodies to periodontopathic bacteria for the development of periodontal tissue destruction is not clarified due to contradictory results of available studies. Whether the antibodies are primarily protective or contribute to tissue degradation is unresolved, and the role of antibodies in type 2 DM is similarly controversial (Zhu and Nikolajczyk 2014). On the other hand, B cells from patients with periodontitis have been shown to produce a pro-inflammatory cytokine profile similar to that of B cells from patients with type 2 DM (Nikolajczyk et al. 2012). Finally, hyperlipidemia seems to interact with DM and periodontitis by increasing the risk of both diseases. The production of pro-inflammatory cytokines is increased by hyperlipidemia, and this may aggravate both insulin resistance and periodontitis (Zhou et al. 2015).

4.2.3 Possible Mechanisms of Association

There are several ways in which DM may influence the periodontal tissues, including cellular activities therein. These involve an impact of DM on the composition of the periodontal microflora, but methodological problems in the studies published so far prevent a firm statement. Thus, it remains unclear whether microbiologic differences between DM patients and controls may be due to the diabetic state, or the result of more severe periodontitis. A recent review concluded that DM and the level of glycemic control have no significant effect on the periodontal microbiota (Taylor et al. 2013b).

Both the periodontal ligament connective tissue and the tooth supportive bone may be affected by processes closely linked to loss of glycemic control. Most important is probably the formation of advanced glycation end products (AGEs) associated with hyperglycemia (Degenhardt et al. 1998). Formation of AGEs is due to a non-enzymatic glycation of proteins and lipids, which results in functional changes in intra- and extracellular proteins. The AGEs formed also imply modified functions of

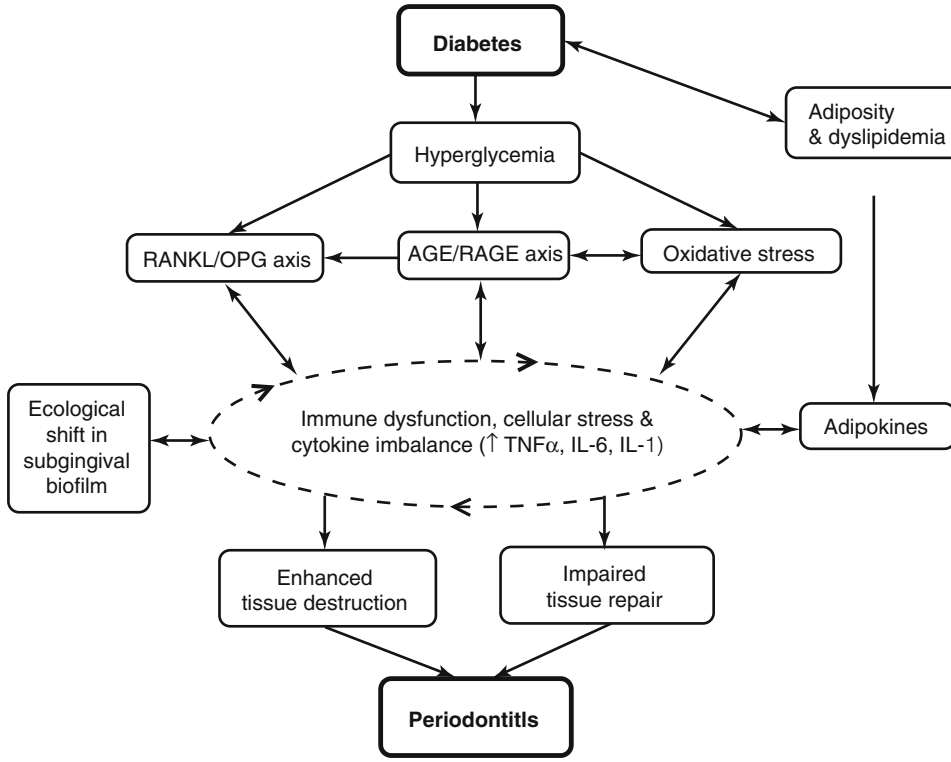

Fig. 4.1 Network of potential mechanisms involved in the pathogenesis of periodontitis in diabetes. The hyperglycaemic state that characterizes diabetes has several deleterious effects. It drives the formation of irreversible advanced glycation end-products(*AGEs*) and the expression of their cheif signalling receptor *RAGE*. This interaction, in turn, leads to immune cell dysfunction, alters phenotype and function of other key cells in the periodontium, and contributes to cytokine imbalance with increased generation of certain pro-inflammatory cytokines. Hyperglycaemia also contributes to enhanced levels of reactive oxygen species (*ROS*) and a state of oxidative stress, both directly and indirectly through the *AGE/RAGE* axis, promoting quantitative and qualitative shifts in cytokine profiles. Finally, hyperglycaemia modulates the *RANKL/OPG* ratio, again directly and indirectly via the *AGE/RAGE* axis, tipping the balance towards enhanced inflammation and destruction. All the above, complemented by the effects of ecological shifts in the subgingival biofilm and the circulating adipokines generated due to diabetes-associated adiposity and dyslipidaemia, drive this vicious cycle of cellular dysfunction and inflammation. The end result is a loss of equilibrium where enhanced periodontal tissue destruction and impaired repair ensue, leading to accelerated and severe periodontitis. Importantly, as shown, several of the associations between the different elements in the figure are bidirectional, for example, the pro-inflammatory state further feeds the generation of *AGEs*, *ROS*, and adipokines, increases the *RANKL/OPG* ratio and helps pathogenic subgingival bacteria thrive. It is also important to note that (a) the amount and quality of evidence supporting the various pathways in this figure varies, and (b) although the goal is to depict the major mechanisms and networks described in the literature, other pathways and links among the various elements shown do exist, but cannot easily be demonstrated in a single schematic. Finally, the processes outlined are potentially modified by several other factors, such as genetics, age, smoking, stress, all of which may contribute significantly to inter-individual variations in disease experience (From Taylor et al (2013a,b). Reproduced with permission from the American Academy of Periodontology, European Federation of Periodontology and John Wiley and Sons)

cells and their receptors. The binding of AGE to one of its receptors (RAGE), the expression of which is increased in DM, may result in synthesis of inflammation-stimulating cytokines, activation of nuclear transcription factor-κB (NF-κB), and production of reactive oxygen species (ROS) (Brownlee 2001), all of which may result in increased cellular apoptosis, reduced bone formation, and increased bone resorption, as thoroughly reviewed recently by Taylor, *Preshaw and Bissett* (2013), Zhu and Nikolajczyk (2014) and Wu, Xiao and Graves (2015). The first mentioned of these reviews presented a model of a mechanism of DM-related bone loss in periodontitis (Fig. 4.1).

The significance of the AGE/RAGE binding has been demonstrated in experimental studies using administration of soluble RAGE, which is the extracellular ligand-binding domain of RAGE, and administration of a RAGE antagonist prevented periodontitis progression in hyperglycemic diabetic mice (Lalla et al. 2000). Also, decreased levels of TNF-α, IL-6, and matrix metalloproteinases (MMPs) in the gingival tissue were found. Other studies have demonstrated that RAGE has fundamental influence on the increased periodontal tissue destruction, which is why antagonists of RAGE have been proposed as a therapeutic tool for the management of DM-associated periodontitis (Lalla et al. 2001). Interaction of AGEs with toll-like receptors (TLRs) has been described, as well as increased expression of TLR2, TLR4, and TLR9 in periodontitis-affected tissues of DM patients, as compared with periodontitis-affected tissue from controls without DM (Rojo-Botello et al. 2012). Further investigations of the TLR-mediated pathways in DM and periodontitis are obviously needed.

B cells, which dominate the inflammatory reaction in the established periodontitis lesion, have been described as a major source of receptor activator of nuclear factor-κB ligand (RANKL) with a pro-osteoclastogenic effect (Onal et al. 2012), and since RANKL expression is increased in mice with type 2 DM (Cao et al. 2010), an exaggerated RANKL expression may potentiate periodontal bone destruction in type 2 DM patients (Zhu and Nikolajczyk 2014). Another source of RANKL is the T cell, but the role of T cells, including whether T-cell produced RANKL plays a role in DM-associated periodontitis, remains to be clarified.

Several studies have investigated the influence of DM on the cytokine profile of patients with periodontitis, and the results reported so far are inconsistent; they are cross-sectional, or they are lacking confirmative support from other studies. Elevated levels of IL-1β in serum and crevicular fluid from DM patients with chronic periodontitis seem to be the most consistent finding (reviewed by Taylor et al. (2013b) and by Atieh et al. (2014)). Studies in animal models have also emphasized the role of TNF-α in prolonging the bacteria-induced immune response in DM-related periodontitis, but evidence from clinical studies is so far inconclusive (Taylor et al. 2013b).

The role of neutrophils in the development of periodontitis, in general, is considered protective, and changes in neutrophil function may account for an increased susceptibility to periodontitis. Indeed, neutrophil function in DM patients with periodontitis has been studied intensively. The outcome of studies based on peripheral neutrophils may conceivably differ from that of neutrophils located in periodontal tissues. However, signs of compromised neutrophil function have been presented in humans, since neutrophil-derived β-glucuronidase and IL-8, which has a chemotactic effect on neutrophils, were depressed in type 2 DM patients with periodontitis (Engebretson et al. 2006). Experimental animal studies in rodent models of DM and/or periodontitis have also revealed reduced neutrophil function (Golub et al. 1982; Sima et al. 2010).

It is well established that hyperglycemia in DM patients may predispose to periodontal tissue destruction, and a large amount of studies have scrutinized the possible pathologic pathways, by which DM may have impact on the course of periodontitis. Fewer studies have dealt with the pathways by which periodontitis may affect the course of DM. High levels of CRP in patients with both diseases have been associated with increased HbA1c levels, and since periodontitis itself may account for higher levels of CRP, the additional systemic inflammation associated with periodontitis may be responsible for the increased HbA1c levels in DM patients with periodontitis (Demmer et al. 2010). Insulin resistance in periodontitis patients with DM may be promoted by hyperreactive neutrophils producing reactive oxygen species, which, in turn, may stimulate pro-inflammatory pathways (Allen and Matthews 2011). An interesting association of periodontal microbiota with prediabetes prevalence in young adults has been described in a recent cross-sectional study (Demmer et al. 2015). Although it is up to future longitudinal studies to determine whether such interrelationships are causal, the finding that levels of potentially periodontopathic subgingival bacteria are abundant in and predictive of prevalent prediabetes is new knowledge (Demmer et al. 2015).

A recent review has focused on the significance of resistin, a biomarker for the levels of which are increased in chronic inflammation including periodontitis. Since resistin has been shown to induce insulin resistance in mice, it has been proposed as a possible link between periodontitis and DM (Devanoorkar et al. 2014).

Several experimental studies in rodents have provided insight in the possible interactions between periodontitis and DM (for review, see Andersen et al. (2007b)). Interestingly, ligature-induced periodontitis has been shown to deteriorate metabolic control in type 2 DM rats with an increase in oral glucose tolerance test of as much as 30 %, and an increase in IL-1β in adipose tissue compared to diabetic rats without periodontitis (Andersen et al. 2006). In prediabetic rats with ligature-induced periodontitis, the glucose tolerance was also significantly impaired, which suggests that periodontitis may facilitate the development of manifest type 2 DM (Andersen et al. 2007a). Moreover, the prediabetic rats with periodontitis developed renal alterations including kidney hypertrophy and a tendency for increased glomerular volume (Andersen et al. 2008).

Although a number of interactions of periodontitis with DM may appear obvious, there is still little evidence to understand the mechanistic pathways of periodontitis' influence on DM, and most is presently speculative.

4.2.4 Outcome of Periodontal Treatment

A large number of studies have examined the role of periodontal treatment for the course of DM, but long-term randomized clinical trials are scarce. The studies are characterized by different inclusion criteria of patients, including various types of DM and various diagnostic criteria for a case of periodontitis. Moreover, stratification for confounders such as smoking, overweight, and medication is difficult. The current evidence has been critically reviewed and analyzed in several papers. A meta-analysis of the outcome of nonsurgical periodontal treatment was performed based on 15 papers selected on the following criteria: randomized controlled study in humans, intervention study on diabetic patients with periodontal disease, minimum 3 months follow-up observation, including data on HbA1c and/or fasting plasma glucose change after treatment, and clear presentation of population demographic data (Corbella et al. 2013). The majority of the patients included in the studies were affected by uncontrolled type 2 DM, and only one study involved patients with type 1 DM. The meta-analyses showed that nonsurgical periodontal treatment significantly reduces the level of HbA1c and fasting plasma glucose in patients with DM. The mean decrease of HbA1c was 0.4 % after 3 months and 0.3 % after 6 months, and the decrease in fasting plasma glucose was 9.0 mg/dL after 3 months and 13.6 mg/dL after 6 months, and there was no positive effect of adjunctive antimicrobials. The authors stated that it was difficult to quantify the clinical relevance of the findings in terms of improved glycemic control. Another meta-analysis of randomized clinical trials included five studies of patients with type 2 DM (Sgolastra et al. 2013). The inclusion criteria were almost similar to the abovementioned, and the primary outcome variables were changes in HbA1c and fasting plasma glucose, while secondary outcomes were changes in total serum cholesterol, serum triglycerides, and high- and low-density lipoprotein cholesterol. The result of the meta-analysis was that the periodontal treatment after 3–6 months resulted in a significant reduction in HbA1c, and in fasting plasma glucose, the mean differences being 0.7 % and 9.0 mg/dL, respectively. Periodontal treatment resulted in no significant differences in the secondary outcomes. This meta-analysis has been criticized for the use of too restrictive exclusion criteria, which may limit the generalizability of the meta-analysis to a fraction of the relevant population (Janket 2014). Finally, a meta-analysis has been presented of the effect of nonsurgical periodontal treatment on systemic inflammation in patients with type 2 DM (Artese et al. 2015). Exclusion of studies due to study design and missing data resulted in four included studies involving associations with CRP and two involving associations with TNF-α, the primary

outcome measures being high sensitivity CRP (hsCRP) or CRP, IL-6, and TNF-α. Adjunctive antimicrobial therapy was combined with scaling and root planing in four of the included studies. A significant reduction as the result of treatment was found for both TNF-α (−1.33 ng/L) and hsCRP (−1.28 mg/L). Taken together, the studies indicate a positive effect on metabolic control and systemic inflammation of nonsurgical periodontal treatment. This is particularly evident in type 2 DM patients. The clinical significance of the improvements obtained, however, is uncertain. Even small reductions in HbA1c may result in significant clinical improvements in diabetic complications and mortality. Thus, for every percentage point decrease in HbA1c, 35 % reduction in microvascular complications has been reported, and an average at 0.2 % point reduction in HbA1c level associates with 10 % lower mortality in type 2 DM patients (UK Prospective Diabetes Study UKPDS Group 1998). The abovementioned reductions in HbA1c levels of 0.31–0.65 % after periodontal treatment, thereby constitutes an important public health benefit. Also, it should be remembered that patients with poor glycemic control may have more insufficient oral hygiene, and they may visit the dentist more infrequently than those with a better blood sugar control, as pointed out by Aggarwal and Panat (2012). This is why special periodontal treatment efforts are recommendable for this group of patients. As mentioned above, a large proportion of type 2 DM and prediabetes patients remain undiagnosed (Glumer et al. 2003; Guariguata et al. 2011), which is a general problem for the prognosis of the patients' health condition in general. However, for the prognosis of the periodontal condition and the result of periodontal treatment, it is very important that these patients are diagnosed as early as possible. An easy and cost-effective way to diagnose type 2 DM is to measure HbA1c level in peripheral blood sampled from the finger (Heianza et al. 2011). Since the majority of adults attend the dental clinic independently of medical treatment needs, and since it is important for the dental treatment to know about diabetic state, it has been proposed to involve dentists in screening of some of their patients for diabetes. In favor of such an arrangement is the fact that the attitude of dentists and their patients is positive to these medical examinations performed in the dental setting (Greenberg and Glick 2012; Greenberg et al. 2012).

Conclusion

The association of periodontitis with diabetes has been described as bidirectional, and there is substantial evidence that poor glycemic control in type 1 and type 2 DM patients is a risk of periodontitis, resulting in increased extension and severity of periodontitis. Due to the global increase in the prevalence of diabetes, the influence of diabetes on the development of periodontitis may be a growing problem. Current evidence also suggests that periodontitis may aggravate the course of DM, but further longitudinal studies are warranted for a firm conclusion to be drawn. The mechanisms by which the two diseases interact are uncertain, but presumably chronic low-grade inflammation enhanced by both diseases plays an important part in the interaction, which obviously involves inflammatory cells and their products, including cytokines and MMPs. The formation of AGE results in modified cellular functions. The existing clinical trials indicate a positive effect on metabolic control and systemic inflammation of nonsurgical periodontal treatment, which may result in a clinically relevant decrease of HbA1c. However, further studies are needed to robustly confirm this.

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Linkage Between Periodontal Disease and Rheumatoid Arthritis

5

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Abstract

The past decades have significantly widened the perspectives of the chronic oral infectious disease known as periodontitis. The disease is regarded as a bacterial infection resulting in low-grade inflammation of the periodontal tissues, and both the associated release of pro-inflammatory mediators and the presence of bacteria in the periodontal pockets, which, as the result of daily procedures, may spread after penetration of the vasculature, are possible mediators of systemic consequences. The present chapter deals with the possible association of periodontitis with rheumatoid arthritis, which may possess a two-way interrelationship.

5.1 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune disease affecting 0.5–1 % of adults in developed countries. The disorder is characterized by persistent synovial inflammation and destruction of joint tissues including the cartilage and bone (Scott et al. 2010). As a consequence, joint deformity occurs, typically affecting the small joints of the hands and the feet where it causes painful swelling. RA may occur at any age, but it usually begins after the age of 40, and women are more often affected than men. Besides joints, the disease may sometimes affect other organs of the body including the skin, lungs, blood vessels, and eyes. An important environmental risk factor is smoking (Klareskog et al. 2009).

Although RA is not regarded as a classical autoantibody-driven autoimmune disease, autoantibodies have been widely used as diagnostic tools. These autoantibodies include rheumatoid factors, which are directed against the constant region of immunoglobulins of the IgG isotype and are present in 70–80 % of the patients with RA (Friswell 2004). Rheumatoid factors occur in many different acute and inflammatory diseases and are thus a rather nonspecific marker of RA. Anti-citrullinated protein antibodies (ACPAs), on the other hand, are also found in 70–80 % of

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patients with RA (Schellekens et al. 2000), and, with a specificity as high as 98 % (Schellekens et al. 2000), they are a more specific marker for RA. Practically all patients with ACPAs have HLA-DRB1 molecules containing the so-called shared epitope capable of binding citrullinated peptides (peptides containing the nonstandard amino acid residue citrulline), which are thought to induce pathogenic T-cell responses, primarily subtypes 0401, 0404, and 0408 in the white population and 0405 in Asians (Nepom and Nepom 1992; Wordsworth et al. 1992). In this subgroup of patients, posttranscriptional conversion of arginine to citrulline, catalyzed by enzymes designated peptidylarginine deiminases (PADs), is regarded an important part in the pathogenesis of the disease (Schellekens et al. 1998). Smoking is thought to mediate release of PADs, which may explain why smoking is a particularly strong risk factor in ACPA-positive patients, who also develop bone erosions earlier and more widely spread than anti-CCP-negative patients. Many investigators therefore consider ACPA-positive and ACPA-negative RA as two different disease entities.

5.2 Association of Periodontitis and RA

5.2.1 Population Data

Several studies have indicated a positive association between periodontitis and RA, sometimes referred to as a bidirectional interaction between the diseases (Cantley et al. 2011). On the other hand, most of the available population studies are small case–control studies providing limited evidence of an association between the two diseases. Some studies have demonstrated that patients with RA are more likely to acquire advanced periodontitis than individuals without RA. This has been shown for young adults (20–35 years) (Havemose-Poulsen et al. 2006) and for midlife to aged people (Käßer et al. 1997; Mercado et al. 2000; Mercado et al. 2001). Based on such findings, it has been proposed to develop systematic programs for prevention of periodontal complications in RA patients (Havemose-Poulsen et al. 2006). However, the outcomes of the many studies are seriously hampered by varying diagnostic criteria for both diseases. In one of the studies on periodontitis, cases were identified on the basis of mean clinical attachment loss being ≥4 mm, and the odds ratio for simultaneous occurrence of RA was as much as 6.09 (95 % CI 1.72–21.55), indicating a strong association although the confidence interval was wide. More extensive cross-sectional studies have provided weaker evidence of an association, the odds ratios being 1.82–1.94, and one of these had ranges of 95 % confidence indicating insignificance (Pablo et al. 2008; Demmer et al. 2011). A 20-year prospective follow-up study including 9,564 American adults defined periodontitis cases by tooth loss of four or more teeth with attachment loss or worse conditions. Baseline and incident cases of RA were defined on the basis of self-reported physician diagnosis or physical examination data corresponding to criteria 1–4 of the American Rheumatism Association 1987 criteria (Arnett et al. 1988). Incident RA was also defined on the basis of death certificate data or health-care facility discharge diagnosis of rheumatism. The adjusted odds ratios for incident RA were between 1.12 and 1.67, dependent on number of missing teeth among participants. Most odds ratios were statistically insignificant, and there was a lack of dose responsiveness (Demmer et al. 2011). A similar result was obtained in a comprehensive 12-year prospective follow-up study in American women (Arkema et al. 2010). Overall, the present data indicating an epidemiological association between periodontitis and RA are inconsistent. The varying case definitions used for both periodontitis and RA may, in part, explain the differences in results obtained. Also, patients with RA are usually receiving intensive anti-inflammatory treatment, which ameliorate periodontal disease progression. The inconsistent results may also be due to an inhomogeneous nature of the patients with the two disease categories, which may both contain patients with more than one disease.

A recent systematic review showed that seven out of ten case–control studies had found

significantly more clinical tooth attachment loss in RA patients compared to controls (Kaur et al. 2013). The same review reported that five of seven studies found significantly increased tooth loss in RA patients compared to controls. When combining the results of the included studies in a meta-analysis, the weighed mean differences were significant in both clinical attachment level and tooth loss between RA patients and non-RA controls. This finding is further supported by a Dutch cross-sectional study, in which a significantly higher prevalence of severe periodontitis in RA patients (27 %) than in controls (12 %) was seen (De Smit et al. 2012). Furthermore, a case–control study compared the presence of severe periodontitis in 287 patients with RA with that in a noninflammatory arthritis control group of 330 patients with osteoarthritis, believed to be demographically similar to the RA group (Mikuls et al. 2014). Anti-cyclic citrullinated peptide antibody-positive patients were significantly more likely to have periodontitis (37 %) than the osteoarthritis controls (26.4 %). A multivariate analysis accounting for confounding factors showed that the anti-cyclic citrullinated peptide antibody-positive patients remained more likely to have periodontitis than controls, the significant odds ratio being 1.59. In this study, tooth loss in the RA patients was also more common than in the control group (Mikuls et al. 2014). Taken together, these studies strongly indicate that the periodontal status is worse in RA patients than in controls (Kaur et al. 2013; Payne et al. 2015). However, there is currently little evidence that periodontitis represents a risk factor for RA (Linden et al. 2013).

5.2.2 Biological Similarities

RA has several clinical and pathological characteristics in common with periodontitis. The diseases, although chronic in nature, show periodical flare-ups with increased tissue-destructive activity in some of the involved sites interposed by periods of relative quietness, and both diseases are quality of life hampering because they are associated with loss of function. Both diseases are characterized by their inflammatory nature with local degradation of collagen-rich soft and hard tissues mediated by cytokines and collagenolytic enzymes. Based on the above findings, it is likely that there is some degree of coexistence, although it is uncertain whether an association is causal or noncausal, for instance, due to shared environmental or other predisposing factors, i.e., smoking, and socioeconomic and genetic risk factors such as MHC class II HLA-*DRB1* (Firatli et al. 1996; Katz et al. 1987; Marotte et al. 2006; Bonfil et al. 1999).

5.2.3 Possible Mechanisms of Association

Both periodontitis and RA have cytokine profiles thought to be involved in the tissue-destructive inflammatory processes, including high production of TNF-α (Cantley et al. 2011). An important example is the common pathway of upregulated expression of receptor activator of nuclear factor κB ligand (RANKL) by fibroblasts and lymphocytes, essential for osteoclast formation (Crotti et al. 2003, Bartold et al. 2010a). Obviously, an exaggerated systemic inflammation induced by periodontal infection might worsen the immune-inflammatory reactions in the joints of RA patients and vice versa (Golub et al. 2006, reviewed by Payne et al. 2015).

In an attempt to identify similarities in the pathology of periodontitis and RA, hematological characteristics of patients with RA have been compared with those of aggressive periodontitis patients. Elevated levels of traditional markers of inflammation could be seen in patients with generalized aggressive periodontitis similar to patients with RA (Havemose-Poulsen et al. 2006). Other case–control studies have compared erythrocyte sedimentation rate, C-reactive protein, ACPAs (measured as autoantibodies to cyclic citrullinated peptides), rheumatoid factor, TNF-α, and interleukin (IL)-1β in RA patients with and without periodontitis, as systematically reviewed by Kaur et al. (2013). The outcome of the studies indicates that there is no good evidence for a correlation between increased levels

of the majority of these factors and presence of periodontitis and RA. An exception is IL-1 level, which appears to be increased in patients with both diseases (Kaur et al. 2013). Moreover, dysregulation of immunoinflammatory responses has been found for both diseases in a number of studies (Mercado et al. 2001; Bartold et al. 2005; Havemose-Poulsen et al. 2005) including similar patterns of elevated IL-10 plasma levels in RA patients and in patients with aggressive periodontitis (Havemose-Poulsen et al. 2005). Thus, gene expression of pro- and anti-inflammatory cytokines in peripheral blood mononuclear cells might be a common denominator for the two diseases, but only few similarities between the two diseases with respect to these parameters have been found (Sørensen et al. 2009). Rheumatoid factors and ACPAs have been revealed in sera from periodontitis patients (Gargiulo et al. 1982; Thé and Ebersole 1991; Havemose-Poulsen et al. 2006), and levels of IgM- and IgA-rheumatoid factors in patients with RA were found to correlate with percentage of sites with clinical attachment loss≥2 mm, which is why these variables have been proposed as possible predictors of periodontal tissue destruction (Havemose-Poulsen et al. 2005) similarly to their use as predictors of joint erosions (Guillemin et al. 2003; Bukhari et al. 2002).

One of the most interesting aspects is the possible involvement of *Porphyromonas gingivalis* in the pathogenesis of RA (Rosentein et al. 2004). In some studies, the frequency of antibodies to *P. gingivalis* has been shown to be significantly higher in patients with RA than in controls (Mikuls et al. 2009; Okada et al. 2011), although this was not the case in another study based on a higher number of patients (Moen et al. 2003). Over recent years, there has been much speculation that *P. gingivalis* may play a role in generation of the citrullinated proteins, which are thought to constitute the pathogenic autoantigens in ACPA-positive patients. *P. gingivalis* has been shown to produce *P. gingivalis* peptidylarginine deiminase (PPAD), which, like human PADs, catalyzes citrullination of proteins. There is no amino acid sequence similarity between PPAD and human PADs, however, and while the bacterial enzyme targets carboxyterminal arginine residues (McGraw et al. 1999) (after cleavage of protein substrates by bacterial gingipains), human PADs efficiently deaminate internal arginine residues (Sugawara et al. 1982). Experimentally, PPAD is capable of citrullinating human fibrinogen and α-enolase, and it has been suggested that immune complexes formed between these citrullinated proteins and ACPAs play a pathogenic role in RA (Wegner et al. 2010). Of note, *P. gingivalis* also expresses an enolase, against the citrullinated form of which ACPAs from patients with RA react (Lundberg et al. 2008). Bacterial enolase might also be citrullinated by human PADs and act as antigens in RA. Indeed, ACPAs have been revealed in inflamed periodontal tissue (Harvey et al. 2013), but direct evidence for a role of *P. gingivalis* in RA remains to be established.

The role of protein citrullination in periodontitis also needs further elucidation. It is noteworthy that the same genetic locus associated with RA and presentation of citrullinated peptides (HLA-DR4) is also associated with severe and rapidly progressive periodontitis, mainly subtypes HLA-DRB1*0401, - 0404, –0405, and –0408 (Bonfil et al. 1999). Increased levels of ACPAs in RA patients with periodontitis compared with RA patients without periodontitis have not been encountered (Pischon et al. 2008).

An essential support for a bidirectional interaction between the two diseases has been established in experimental studies. Adjuvant arthritis was induced in rats, some of which were subsequently systemically treated with tissue inhibitor of matrix metalloproteinases (TIMP-4). At 3 weeks the rats were examined for signs of periodontitis. Rats untreated with TIMP-4 showed significantly increased periodontal bone loss and tooth mobility, which was improved in rats treated with TIMP-4 (Ramamurthy et al. 2005). In another experimental study, induction of arthritis in mice with preexisting periodontitis resulted in exacerbation of arthritis, as compared to mice without periodontitis (Cantley et al. 2011). Further evidence for a relationship between inflammation and a periodontitis-associated pathogen was provided in another

experimental study in rats. These animals had foam pieces loaded with heat-killed *P. gingivalis* implanted in their backs with subsequent induction of adjuvant arthritis. The study showed that severe arthritis developed more rapidly in rats with preexisting *P. gingivalis*-induced inflammatory lesions distant from the joints than in controls (Bartold et al. 2010a, b).

5.2.4 Outcome of Periodontal Treatment

Several studies have examined the effect of periodontal treatment on biomarkers and course of RA, as systematically reviewed and meta-analyzed by Kaur et al. (2014). As an example, full-mouth scaling and root planing resulted in reduced erythrocyte sedimentation rate after 3 months in 26 patients with RA, but there was no significant effect on the degree of disability or IgM-rheumatoid factor level (Ribeiro et al. 2005). Another clinical trial including 40 patients with moderate to severe RA, receiving either disease-modifying antirheumatic drugs alone or in combination with anti-TNF-α, had nonsurgical periodontal treatment. After 6 weeks the periodontal treatment had a beneficial effect on signs and symptoms of RA (Ortiz et al. 2009). Based on the 12 articles included, Kaur et al. (2014) concluded that the sample sizes of the available studies were small and the duration of the studies was limited. Nonetheless, the studies provided support for the hypothesis that periodontal infection control by nonsurgical periodontal treatment could reduce clinical and biochemical markers of active RA. Larger studies with a longer duration are needed to fully understand whether periodontal treatment has an effect on disease activity in RA patients.

Another new interesting perspective is the similar effect of matrix metalloproteinase inhibitors in periodontitis and RA patients. This effect, which appears to be synergistically enhanced combined with an anti-inflammatory drug, is supposed to be due to a local effect in the affected tissues and due to a reduced systemic inflammation (reviewed by Payne et al. (2015)).

Conclusion

Several studies have demonstrated an association of periodontitis and RA. There is ample evidence of similarity in the pathogenesis of the two diseases, and a large body of studies has shown that patients with RA suffer more periodontal attachment loss. This is why it has been proposed to develop systematic programs for prevention of periodontal complications in RA patients. Some short-term intervention studies have also shown that periodontal treatment may reduce the disease activity in patients with RA, but larger studies with a longer duration are warranted, and currently there is little evidence that periodontitis represents a risk factor for RA. A possible involvement of *P. gingivalis* in the pathogenesis of RA via citrullination of proteins remains to be further evidenced.

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6 Association Between Dental Infections and Renal and Liver Diseases

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Abstract

The number of patients with kidney and liver diseases is increasing due to global aging of populations, increased obesity leading to metabolic syndrome and diabetes, and behavioral factors such as alcohol consumption, and contagious diseases such as hepatitis virus infections. Oral and dental infections may have detrimental effects on the course and treatment of these diseases and should thus be diagnosed and properly treated. The end stage of both kidney and liver diseases calls for organ transplantation and hence lifelong immunosuppression. This renders the patient liable for all kinds of infections. In these patients, insidious dental infections can turn out to be life threatening.

6.1 Introduction

The kidneys play a major role in body homeostasis by filtering metabolic waste products, and they are also involved in a number of critical processes such as regulation of electrolyte balance, blood pressure control, and stimulation of the red blood cell production by erythropoietin. The global prevalence of chronic kidney disease is estimated to be 8–16 % (Jha et al. 2013). Serious problems arise when the kidney function drops so that less than 25 % of the function remains. Stages of chronic kidney disease are assessed using the glomerular filtration rate (GFR) where values below 15 ml/min/1.73 m^2 mean kidney failure. Severe renal diseases are treated with dialysis and finally with kidney transplantation.

The liver, respectively, is the largest internal organ and no human can survive without it. Liver helps in digestion by producing bile for lipid metabolism, but liver also is the principal chemical factory of the body. It synthesizes proteins, such as albumin, hormones, and blood coagulation factors and is responsible for glucose metabolism and storage for many vitamins. The liver also detoxifies a number of harmful substances such as alcohol, bacterial toxins, and many drugs. The renin-angiotensin system is an example of the interplay between the liver and kidneys.

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Liver failure leads to the death of the patient due to multi-organ failure. There are a number of etiologic causes for liver diseases such as viral hepatitis and extensive alcohol consumption, to mention just two. Liver function can be assessed by several clinical chemistry parameters, for example, by serum glutamyltransferase. Liver cirrhosis is the result of death of functioning hepatocytes which leads to fibrosis. Its incidence is rising in the industrialized countries mainly due to obesity and alcohol consumption. For example, in the USA, death rate in liver cirrhosis in 2012 was 14.9 in men and 7.1 in women per 100,000 individuals (WHO Global Information System on Alcohol and Health 2014). The ultimate treatment of liver diseases is organ transplantation.

6.2 Renal Diseases

The global increase in diabetes directly reflects in the increase of renal diseases because of diabetic nephropathy. End-stage renal failure is also mainly caused by diabetes (Atkins 2005). In addition to diabetic nephropathy, there are several other etiologic factors for kidney disease. Chronic glomerulonephritis and polycystic kidney disease usually are slow-progressing diseases, but there are many other heterogenic renal diseases, such as nephrosclerosis, arteriosclerotic nephropathy, urinary tract obstruction, tubulointestinal nephritis, renal amyloidosis, and congenital or hereditary kidney diseases.

Periodontal disease is prevalent in patients with chronic kidney disease (Akar et al. 2011; Chambrone et al. 2013). Patients with diabetic nephropathy have shown particularly poor oral health compared with patients with glomerulonephritis which finding supports the known two-way relationship between oral health and diabetes (Teratani et al. 2013; Preshaw et al. 2012). Periodontal pathogens have also been associated with chronic kidney disease (Niedzielska et al. 2014; Ismail et al. 2015). Oral health is often poor among patients with chronic kidney disease (Vesterinen et al. 2011). Periodontitis also reflects in low serum albumin concentration in end-stage kidney disease (Kshirsagar et al. 2007). This in turn is a marker of mortality, and albumin can also be measured from saliva samples of the patients (Meurman et al. 2002).

6.3 Streptococcal Glomerulonephritis

Before the advent of penicillin, streptococcal glomerulonephritis was highly prevalent (Nasr et al. 2013). Today it is rare in industrialized countries with estimates between 9.5 and 28.5 new cases per 100,000 individuals per year (Rodriguez-Iturbe and Musser 2008). Glomerulonephritis nevertheless is one of the two types of infections caused by *Viridans streptococci* – the other being endocarditis. These two disease entities often occur concomitantly, and rheumatic fever should be mentioned in this context (Neugarten and Baldwin 1984). Hence, infections of the mouth and teeth have long been known to be important causative factors of this renal disease. The caries bacterium *Streptococcus mutans* has also been associated with glomerulonephritis (Okada et al. 1996). A causal link between periodontitis and glomerulonephritis has similarly been suggested (Ardalan et al. 2011). It is thus evident that maintaining good oral health is important in such patients. Administering prophylactic antibiotic before dental treatment to patients with history of glomerulonephritis is still recommended even though scientific evidence for this practice is weak (Del Mar et al. 2004).

6.4 Liver Diseases

Apart from metastatic oral infections and case reports of patients with liver abscesses from dental origin, there is hardly any literature on the role of oral infection in liver diseases (Gendron et al. 2000; Kajiya et al. 2008). However, oral health status of patients with chronic liver disease is known to be poor in general, and many patients suffer from xerostomia (Guggenheimer 2009; Helenius-Hietala et al. 2013a, b). Xerostomia is often associated with salivary gland hypofunction, and it has been shown that liver transplant recipients have

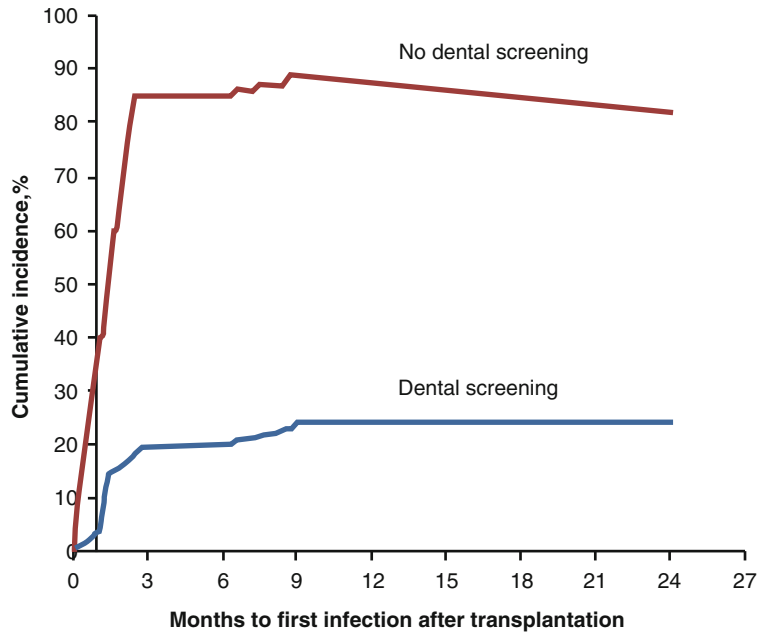

Fig. 6.1 Dental infections associated with the incidence of complications after liver transplantation. Patients who had no dental examination and treatment because of emergency operation had more posttransplant complications than those whose dental problems had been treated (Modified from Helenius-Hietala et al. 2013)

low whole saliva flow rates, which lead to further deterioration of the oral health (Helenius-Hietala et al. 2013a, b).

Helenius-Hietala *et al.* (2013a, b) have investigated liver transplant patients and observed increased risk for posttransplant infection complications in those patients by whom no dental treatment had been given before the operation because of lack of time, in comparison to those with more elective transplantation: OR 8.17 (95 % CI 2.19–30.6). Similarly, the assessed need for dental extractions was found to associate with reduced time from diagnosis of liver disease to the need of transplantation and the number of tooth extractions correlated significantly with change in the Model for End-Stage Liver Disease (MELD) score (Fig. 6.1) (Aberg et al. 2014). In the same study, *Streptococcus viridans* was detected in peritonitis cases only among the patients with dental infections.

Nagao *et al.* (2014) reported that periodontal disease may worsen the progression of liver disease caused hepatitis virus infection by reducing platelet count, for example, with OR 5.80 (95 % CI 2.30–14.92). The periodontal pathogen *Porphyromonas gingivalis* has also been linked to the progression of liver disease in patients with nonalcoholic fatty liver (Yoneda et al. 2012). In addition, periodontitis has been shown to be associated with hepatocellular carcinoma (Tamaki et al. 2011). Periodontitis may further enhance alcohol-induced liver damage as shown in an animal experiment (Tomofuji et al. 2008). The importance of lipid and sugar metabolism of the liver was further emphasized in a study where periodontitis was associated with hepatic steatosis (Saito et al. 2006). In this investigation, the severity of periodontitis increased with elevated serum values measuring liver function.

Conclusion

Scientific evidence of the role of oral infections in renal and liver diseases is still weak. However, it is known that chronic oral infections such as periodontitis affect many systemic metabolic pathways and by causing endothelial dysfunction, for example, which is detrimental in all organs (Janket et al. 2008). Studies have shown that if eradication of dental infection foci has been neglected, the outcome of patients with kidney or liver disease may be compromised. Hence, maintaining good oral health and treating infection foci properly is highly important also in these patient groups (Table 6.1).

Table 6.1 Aspects of oral infections in patients with kidney and liver diseases

Chronic kidney disease	Liver disease
Streptococcal glomerulonephritis is the type of infection often caused by *Viridans streptococci*	Dental infections may cause liver abscesses
Periodontal disease is prevalent among the patients	Xerostomia affects detrimentally oral health
Periodontal pathogens have been associated with chronic kidney disease	Poor dental health associates with complications in liver transplantation
Treating oral and dental infections necessary before dialysis/kidney transplantation	Treating oral and dental infections necessary before liver transplantation

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Association Between Oral Infections and Cancer Risk

7

Jukka H. Meurman

Abstract

Highly prevalent dental infections have been shown to statistically associate with cancer. Periodontal disease in particular links to head and neck cancer but also to malignancies in other organs. Metabolism of oral microorganisms may lead to carcinogenic substance that also affect oral mucosa locally thus posing a risk for oral cancer. However, scientific evidence is still weak in these associations.

7.1 Introduction

Cancer is always characterized by infection, but infection may also be causally linked to the development of malignancy (zur Hausen and de Villiers 2014). Classical examples are certain human papillomavirus (HPV) infections and cervical cancer and *Helicobacter pylori* infection and gastric cancer. In general, chronic inflammations induced by microorganisms are considered important in the carcinogenesis (Kuper et al. 2000). Recently, also bacterial and yeast infections of the mouth have been statistically associated with the development of cancer in various organs (Meurman and Bascones-Martinez 2011; Söder et al. 2015). Oral microbiome may also play a role in oral cancer where, for example, local acetaldehyde production by oral microorganisms has been shown to pose a marked risk (Kurkivuori et al. 2007; Meurman 2010). However, in general there is a long latency between the initial infection and tumor appearance, and not at all infected person develops cancer.

7.2 Oral and Dental Infections and Cancer Epidemiology

One unique characteristic in oral and dental infections is their mostly chronic nature and very high prevalence in populations. Dental caries is regarded as one of the most widespread infection of humans in the world. Also the prevalence of periodontal disease is very high. The World Health Organization (WHO) has estimated that 60–90 % of schoolchildren and nearly 100 % of adults worldwide have dental caries, and, respectively, 15–20 % of 35–44-year-old adults have severe periodontal disease (Petersen et al. 2005, WHO

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Oral Health 2012). Both caries and periodontal disease are multi-bacterial infections caused by the oral biofilm (dental plaque). Recent studies have shown that oral microbiota is far more complex and numerous in species than hitherto understood. It may, in fact, comprise thousands of microbial species (Keijser et al. 2008). Studies have also been conducted analyzing the microbiota at the site of oral cancer compared with normal mucosa; in this, for example, the number of streptococci was decreased in cancer specimens (Schmidt et al. 2014). Correspondingly, saliva may also reflect the diversity of microbiota in patients with oral cancer (Pushalkar et al. 2011). Dental bacteremia on the other hand is common, and thus oral microbes easily gain access to blood circulation and may then cause systemic complications (Lockhart et al. 2008) and possibly link to cancer in general (Meurman 2010).

Oral yeast infections need to be mentioned here. The global prevalence of *Candida* infections is not known. In certain patient groups such as human immunodeficiency virus (HIV) infected, the prevalence can be 90 %, and WHO has estimated that approximately 9.5 million people would be infected with *Candida* (The Fungal Research Thrust 2011). *Candida* is more prevalent on dysplastic and carcinoma lesions than on healthy oral mucosa (McCullough et al. 2002). The role of *Candida* in carcinogenesis is particularly evident in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), which is a genetic, autosomal recessive disorder. These patients frequently present oral and esophageal carcinomas (Rautemaa et al. 2007). The mechanisms how *Candida* links to the development of cancer may be partly due to its invasiveness. Degradation of the epithelial basal membrane components and disruption of cell-to-cell contacts have been shown in experiments with *Candida* (Parnanen et al. 2008, 2010).

As regards cancer, this is among the leading causes of morbidity and mortality in the world. The WHO reported approximately 14 million new cases and 8.2 million cancer-related deaths in the year 2012 (WHO Cancer 2015). Furthermore, the number of cases with malignancies is estimated to increase by 70 % by the year 2030 because of aging of the populations. Hence if oral infections play any part in the development of cancer, or modify the process of carcinogenesis, the association here discussed is evidently of high importance. Namely, these easily preventable diseases (in particular caries and periodontitis) should then be better controlled.

7.3 Infection-Driven Mechanisms in Carcinogenesis

In carcinogenesis cells accumulate changes in the genetic material which modify their function. Cell proliferation, differentiation, senescence, and apoptosis are involved in the regulation of the cell cycle, and all these functions may be involved in carcinogenesis (Lundberg and Weinberg 1999). Infection and inflammation, in turn, may interfere with cell metabolism and functions causing upregulation of a number of cytokines and inflammatory mediators, which trigger cascade-like reactions further leading to DNA damage, impaired DNA repair, mutations, and uncontrolled cell proliferation (Chang and Parsonnet 2010). Infection-driven carcinogenesis thus involves several mechanisms. These include inflammation caused by microbial infection, lymphoproliferation, infection-induced hormonal changes that affect epithelial cell proliferation, cell transformation directly caused by infection, and toxic and carcinogenic mechanisms of the microbes in question (Chang and Parsonnet 2010). These pathways are depicted in Fig. 7.1.

7.4 Acetaldehyde Production by Oral Microorganisms

Alcohol is not carcinogenic but the first metabolite of ethanol, acetaldehyde, is highly carcinogenic. Although the liver is the organism responsible for 75–90 % of ethanol metabolism, extrahepatic pathways also exist. Ethanol is oxidized also by mucosal and microbial cells yielding acetaldehyde, formed by alcohol dehydrogenase enzyme. Acetaldehyde is further metabolized by aldehyde dehydrogenase yielding acetone which is less toxic and less harmful

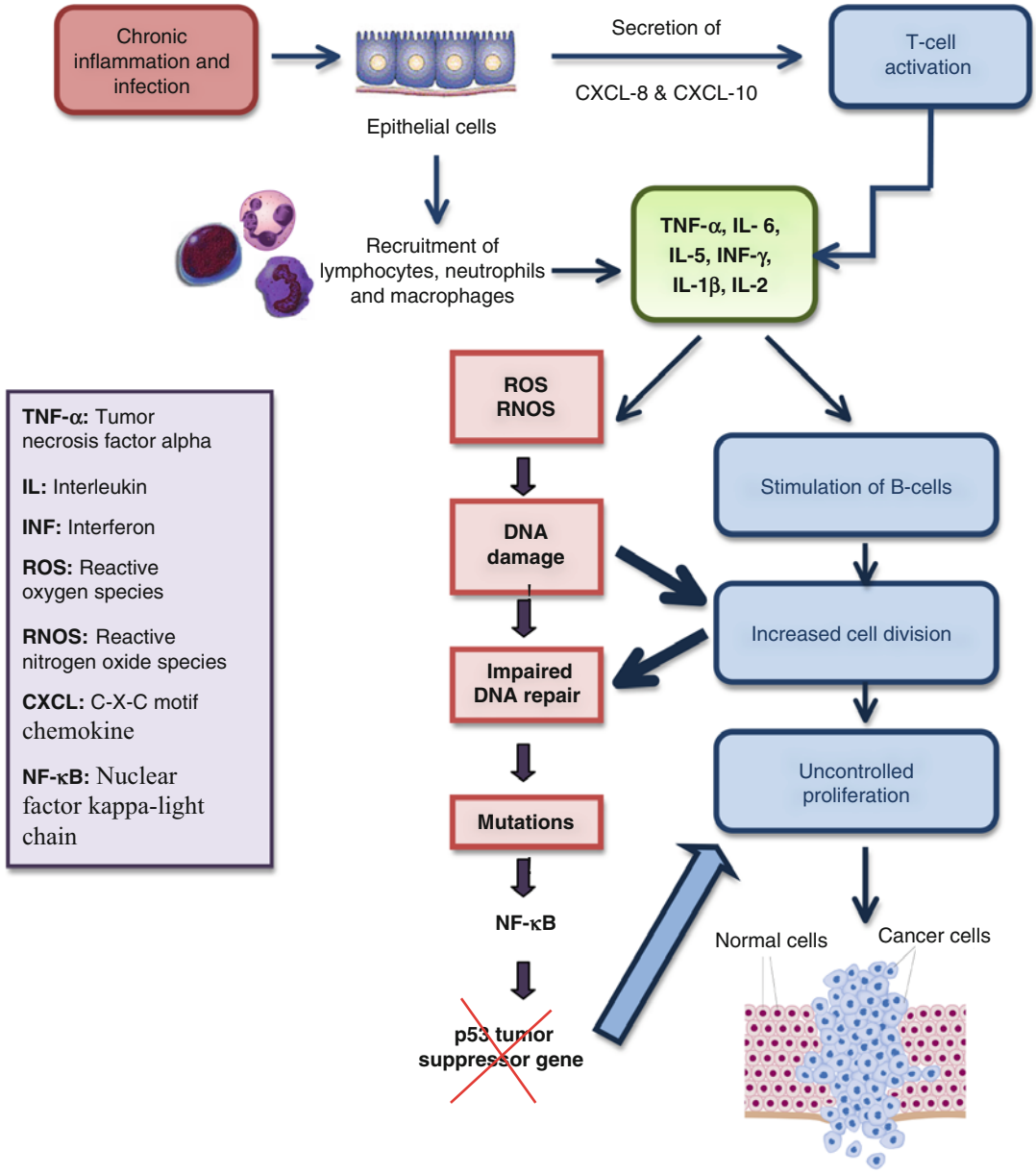

Fig. 7.1 Metabolic pathways in infection-driven carcinogenesis (Picture prepared by Bascones-Martinez)

compound. This, in turn, is oxidized to carbon dioxide and then eliminated from the body.

Homann et al. (1997) were the first to show high levels of acetaldehyde in saliva after intake of alcohol. This group further showed that drinking alcohol and smoking concomitantly increased salivary acetaldehyde concentrations and that poor oral hygiene associated with this risk (Homann et al. 2000, 2001) (see Fig. 7.2). Later, also oral *Candida* species were shown to produce acetaldehyde from ethanol partly explaining why *Candida* infections as such have been linked to oral cancer (Nieminen et al. 2009; Uittamo et al. 2011). Moritani et al. (2015) reported that indeed considerable numbers of oral bacteria have the capability to produce acetaldehyde from ethanol. Today it seems clear that the ethanol metabolism here discussed

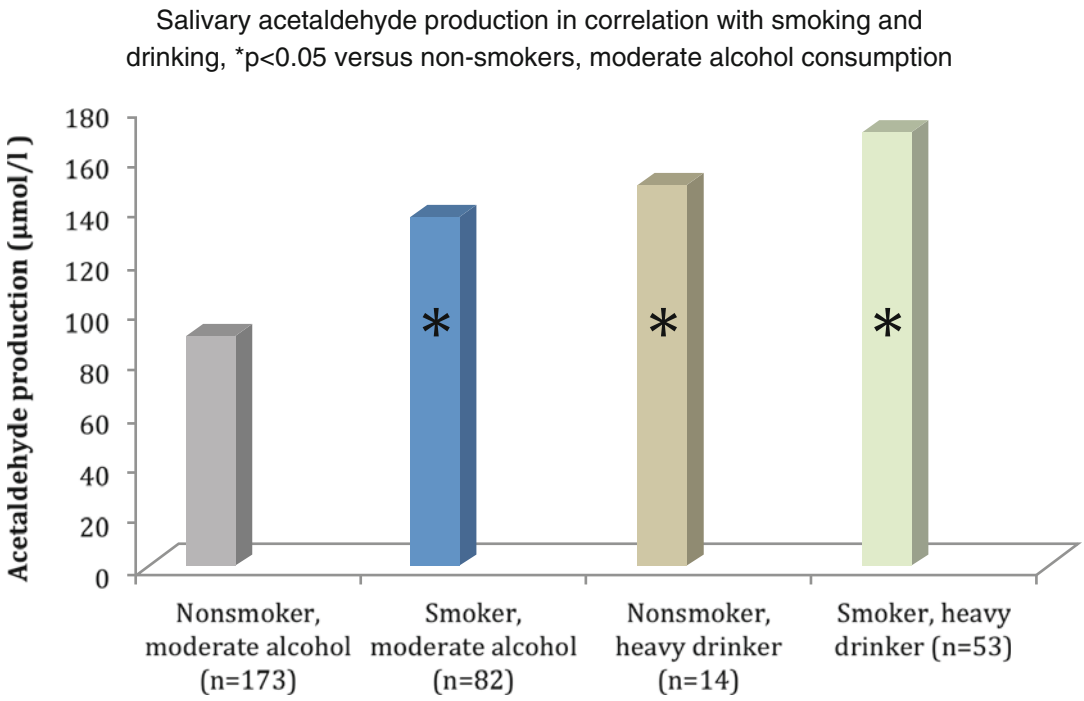

Fig. 7.2 Relationship between drinking alcohol and smoking and salivary acetaldehyde concentration (Modified from Homann et al. 2000)

is an evident pathologic mechanism in the development of oral and upper gastrointestinal tract cancer.

7.5 Caries, Periodontitis, and Cancer

Chronic periodontitis in particular associates with oral cancer risk. In a large study from the USA comprising more than 13000 subjects, clinical attachment loss, a proxy for periodontitis, associated with the presence of tumor (OR 4.57, 95 % CI 2.25–9.30) and premalignant lesions (OR 1.55, 95 % CI 1.06–2.27), respectively (Tezal et al. 2005). Periodontitis was found to also associate with tongue cancer risk (OR 5.23, 95 % CI 2.64–10.35) (Tezal et al. 2007). Concomitant HPV infection seems to play a role in this regard too (Tezal et al. 2009).

As regards dental caries, there might be an inverse relationship between this dental disease and cancer. Tezal et al. (2013) investigated in a case–control study how cardiological status parameters link to head and neck cancer. It appeared that caries lesions showed an OR 0.55 (95 % CI 0.30–101) regarding cancer. The authors suggest that the lactobacilli prevalent in caries lesions might exert beneficial effect and enhance the immune system against cancer.

Virtanen et al. (2014), on the other hand, showed in their observational study of 1390 subjects with 24 years of duration that dental infections in periodontally healthy subjects associated with the incidence of any cancer (OR 2.62, 95 % CI 1.18–5.78). Gingivitis was also shown to link to cancer in this same Swedish cohort study (Söder et al. 2015). Furthermore, after 26 years of observation in the same study, high gingival index score associated with the incidence of any cancer with OR 1.29 (95 % CI 1.00–1.65). The statistical association between oral infections and cancer has also been observed specifically with certain types of cancer. Söder et al. (2011) found in their cohort that missing any molar tooth from the mandible

associated with incidence of breast cancer with OR 2.36 (95 % CI 1.07–5.21). Missing molars were the proxy for history of dental infections. It is evident, however, that more studies are needed for final conclusion regarding the associations between caries, periodontal disease, and cancer.

7.6 Role of Saliva in the Oral Infection-Linked Carcinogenesis

Little is known about the role of saliva in oral infection-related carcinogenesis. It has been observed that salivary characteristics differ in patients with and without head and neck tumors so that saliva form those with malignancy showed more cytotoxic effect on fibroblasts than that from healthy controls (Bloching et al. 2007). Oral microorganisms may also metabolize dietary components into carcinogenic substances. Saliva may contain nitrosamines, for example (Bahar et al. 2007). However, more studies are called for before any conclusions can be drawn.

Conclusion

The highly prevalent oral infections and dental diseases in particular pose threat to systemic health if not diagnosed in time and properly treated. Studies have shown that statistical associations exist between chronic dental infections and development of cancer. Head and neck and upper gastrointestinal tract cancer may be affected by direct oral microbial metabolism leading to carcinogenic substances thus triggering malignant development in the tissue. But malignancy in any organ may in fact be affected by infection.

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Oral Candidiasis and the Medically Compromised Patient

8

Camilla Kragelund, Jesper Reibel, and Anne Marie Lynge Pedersen

Abstract

Oral candidiasis is a common opportunistic oral infection in humans caused by overgrowth of *Candida* species, in particular *Candida albicans*. Clinically it usually presents as pseudomembranous or erythematous candidiasis. It may be asymptomatic or associated with local discomfort, dysgeusia and xerostomia. The most common risk factors for oral candidiasis include treatment with antibiotics, poor oral hygiene, tobacco smoking, denture wearing and salivary gland hypofunction. A large number of diseases as well as their treatment including diabetes, cancer and cancer therapy, HIV infection and treatment with immunosuppressants are associated with oral candidiasis. In immunocompromised patients, the localized oral infection can spread through the bloodstream or upper gastrointestinal tract leading to severe infection with increased morbidity and mortality. This chapter focuses on *Candida* as commensal oral microorganism, the clinicopathological aspects in medically compromised patients and diagnostic methods available regarding oral candidiasis.

8.1 Introduction

Candida species, in particular *Candida albicans*, are part of the normal oral microbiota. The percentage of carriers varies considerably in different studies due to geographical variations and variations in subjects examined, sampling methods and identification techniques. Possibly, 30–50 % of healthy individuals harbour *Candida* species as part of their oral microbiota (Odds 1988), and it is likely that *Candida* plays a role in maintaining a balance between microorganisms and the host (Krom et al. 2014).

C. albicans exists in different morphological forms: blastospores, yeast form and filamentous forms, pseudohyphae and true hyphae; where pseudohyphae appearing to be an intermediate between blastospores and true hyphae (Carlisle

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et al. 2009). The yeast form is occurring in the normal oral microbiota, although hyphae may be seen in the absence of infection (Arendorf and Walker 1980; Rindum et al. 1994). In general, however, the filamentous forms are related to invasive infection in the oral mucosa (Carlisle et al. 2009). Thus, the ability of the fungus to transform from blastospores to hyphae is important for the virulence of *C. albicans*. Interestingly, a salivary component, statherin, seems to be able to induce the inverse transition (Leiro et al. 2009). Another important ability of *C. albicans* is "phenotypic switching" (Soll et al. 2014) that may enable the fungus to evade the immunological defence and even adapt to antifungal agents and thereby increase virulence.

Apart from *C. albicans*, other *Candida* species such as *C. glabrata*, *C. krusei* and *C. tropicalis* have been isolated from healthy individuals (Zaremba et al. 2006). *C. dubliniensis* is an emerging species initially recovered from patients infected with human immunodeficiency virus (HIV) (Sullivan et al. 1995). Recent studies identified *Candida/Pichia*, *Cladosporium/Davidiella*, *Alternaria/Lewia*, *Aspergillus/Emericella/Eurotium*, *Fusarium/Gibberella*, *Cryptococcus/Filobasidiella* and *Aureobasidium as* consensus genus-level members of the basal human salivary mycobiome from healthy human mouth using multitag pyrosequencing of panfungal internal transcribed spacer (ITS) primers and massive parallel, high-throughput sequencing of ITS1 amplicons from saliva (Ghannoum et al. 2010; Dupuy et al. 2014). *Saccharomyces*, *Epicoccum* and *Phoma* were weaker candidates for consensus inclusion. However, *Malassezia* species were included in the oral core mycobiome, which is interesting since they are important commensals/pathogens of human skin (Dupuy et al. 2014).

Infection requires recognition and adhesion to epithelial cells, and following subsequent multiplication and secretion of extracellular matrix, a biofilm can form on the mucosal surfaces (Cannon et al. 1995). Hyphae formation is important for formation of a stable biofilm; thus, hyphal growth is important for the virulence of *C. albicans*. Secretion of enzymes such as proteases and lipases facilitates tissue penetration and furthermore degrades immunoglobulins which help to evade the host defence. Interestingly, several studies have shown that bacteria coexist with *C. albicans* in oral biofilms (Budtz-Jørgensen 1990), and it seems that this influences the growth and virulence of *C. albicans* (Thein et al. 2006, 2009; Diaz et al. 2014; Cavalcanti et al. 2015). Thus, a symbiotic relationship between *Streptococcus mutans* and *C. albicans* has been shown to synergize virulence of dental plaque biofilms in vivo (Falsetta et al. 2014). Furthermore, *Streptococcus gordonii* glucosyltransferase promotes biofilm interactions with *C. albicans* (Ricker et al. 2014).

In order to maintain in the oral cavity, the fungi not only need to grow, reproduce themselves and bind to a surface, they also have to resist the antimicrobial activity of saliva. Saliva exhibits antimicrobial activity by killing and inhibiting growth but also by preventing adhesion and colonization to the surfaces in the oral cavity. Moreover, saliva contains numerous antimicrobial proteins and peptides of which histatins, especially histatin 5, are the most important ones regarding antifungal activity. Histatins are small molecular weight proteins produced by the human salivary glands, which exhibit fungicidal and fungistatic activities against *C. albicans* and other *Candida* species like *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. lambica*, *C. parapsilosis*, *C. pseudotropicalis*, *C. stellatoidea and C. tropicalis*, as well as *Saccharomyces cerevisiae* and *Cryptococcus neoformans* isolated from healthy and immunocompromised patients (Oppenheim et al. 1988; Tsai and Bobek 1998; Xu et al. 1991). Other important salivary proteins with antifungal properties include lactoferrin and lysozyme (Samaranayake et al. 2001). The importance of the salivary antifungal activity becomes evident in cases of immune deficiency and disease- and/or medication-induced salivary gland hypofunction being associated with high rates of oral candidal carriage and *Candida* infections (Costa et al. 2006; Lam et al. 2012; Lin et al. 1999; Pedersen et al. 2015; Shiboski et al. 2015; Yan et al. 2011).

8.2 Clinicopathological Aspects

In patients carrying *Candida* as part of the commensal oral microbiota, overgrowth of *C. albicans* or non-albicans *Candida* spp. can cause oral candidiasis (Rindum et al. 1994). The diagnosis oral candidiasis is based on clinical signs and/or oral mucosal symptoms together with positive test results compatible with candidal overgrowth. Characterization of oral candidiasis according to sporadic or recurrent infection, duration of infection (acute/chronic), symptomatic or asymptomatic infection, primary or in relation to other oral or systemic diseases (primary/secondary/tertiary) is important in unravelling and managing oral candidiasis. Most patients experience sporadic candidal infection, whereas less experiences multiple recurrent infections. Information from medical history and clinical evaluation are used to classify acute or chronic candidiasis as no arbitrary time limit makes clinical sense as individual host and oral environmental factors influence the course of the infection. Subjective symptoms in relation to candidiasis are usually associated to the clinical type. Oral candidiasis presents clinically in various forms such as pseudomembranous, erythematous and hyperplastic candidiasis (Ellepola and Samaranayake 2000). It is still largely unknown why oral candidiasis manifests in these different variants in different individuals (Reichart et al. 2000). Furthermore, there are candida-associated lesions which further complicate the diagnosis.

Pseudomembranous candidiasis is characterized by thick, white patches covering part of the mucous membranes often being the soft palate, tongue and buccal and lip mucosa (Fig. 8.1). The pseudomembranes can be wiped off easily. In chronic infections the mucous membrane underneath often is erythematous with pinpoint haemorrhages. Normally no soreness is associated with the pseudomembranous type, but taste disturbances as salty or metallic taste are reported. Acute neonatal thrush is common as a result of favourable candidal growth conditions because of immature oral microbiota and oral immunity. Chronic pseudomembranous candidiasis can be seen in immunocompromised patients and in relation to topical exposure to steroids, e.g. asthma inhalators.

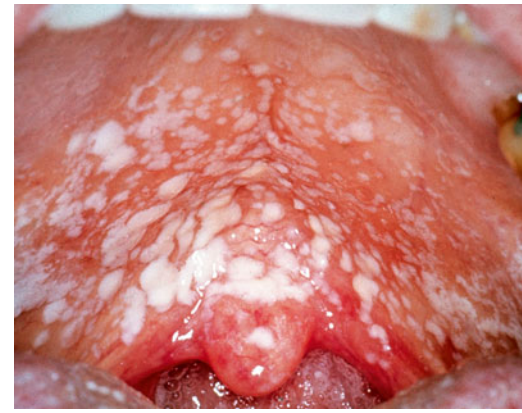

Fig. 8.1 Pseudomembranous candidiasis in the soft palate

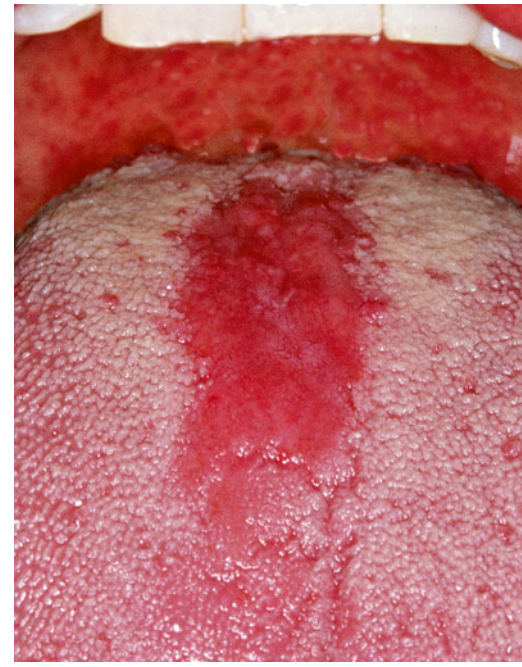

Fig. 8.2 Chronic erythematous candidiasis on the dorsal surface of the tongue ("median rhomboid glossitis")

Erythematous candidiasis is characterized by unspecific focal or generalized redness of the mucous membranes (Fig. 8.2) and is often associated with symptoms as burning and stinging sensation. Antibiotics are the most common cause of acute primary erythematous candidiasis.

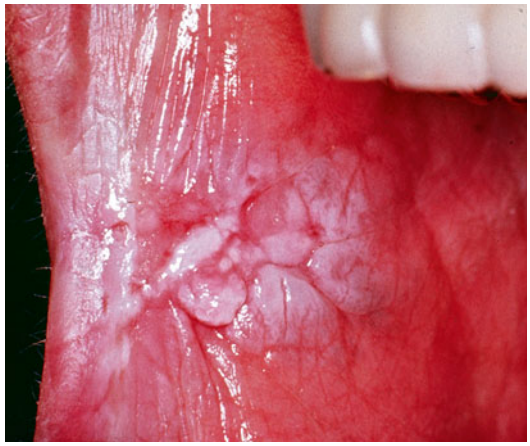

Fig. 8.3 Chronic hyperplastic candidiasis in the anterior part of the right buccal mucosa

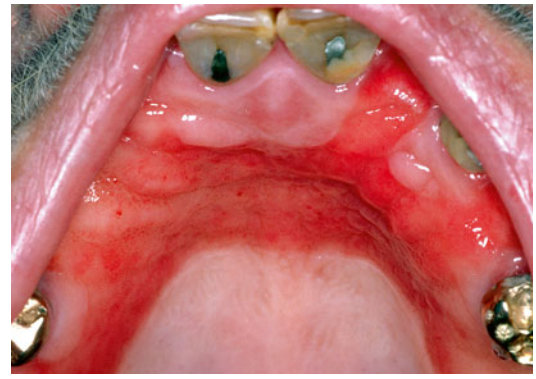

Fig. 8.4 Chronic erythematous candidiasis presenting as denture stomatitis

Chronic secondary erythematous candidiasis is common in oral lichen planus.

Hyperplastic candidiasis (Fig. 8.3) is a chronic infection and has two primary manifestations. The nodular type presents as small white slightly elevated papular lesions giving the mucous membrane a speckled appearance. The plaque-like type is homogenous white slightly elevated areas of the mucous membrane. It is often related to smoking and seen in the commissural area of the buccal mucosa. The hyperplastic lesions cannot be rubbed off and are usually asymptomatic. The two types may occur simultaneously. The hyperplastic types are to some extent controversial as they may represent secondary candida infections in leukoplakias (Holmstrup and Bessermann 1983), and the term candidal leukoplakia is sometimes used (Sitheeque and Samaranayake 2003). A biopsy is generally indicated to exclude malignancy, and the treatment result after antifungal therapy should always be monitored.

Candida-associated lesions including denture stomatitis (Fig. 8.4), angular cheilitis, median rhomboid glossitis (Fig. 8.2) and linear gingival erythema may all be associated with *Candida* infection but may also be related to other causes, e.g. ill-filling dentures and bacterial or mixed bacterial and fungal infection. Thus, a thorough investigation is mandatory before adequate treatment can be initiated. In particular it is important to rule out a premalignant or malignant lesion, e.g. leukoplakia, erythroplakia or oral cancer, secondarily infected with *Candida*, or a disorder needing other management or supplementary treatment, e.g. lichen planus or lupus erythematosus (Table 8.1).

Table 8.1 Different types of oral candidiasis and differential diagnostic considerations

Oral candidiasis	Differential diagnostic considerations
Pseudomembranous	No similar lesion
Erythematous	Erythematous oral lichen planus, erythroplakia, oral cancer
Hyperplastic	
Nodular	Non-homogeneous leukoplakia, oral cancer
Plaque	Homogeneous leukoplakia
Denture stomatitis	Ill-fitting dentures, poor denture hygiene
Angular cheilitis	Bacterial infection, malnutrition, vitamin and/or mineral deficiency
Median rhomboid glossitis	Geographic tongue, malnutrition, vitamin and/or mineral deficiency
Linear gingival erythema	Gingivitis (bacterial)

8.2.1 Histopathology

Candidal hyphae are readily identified in biopsies by the use of an appropriate staining method, e.g. Periodic Acid-Schiff (PAS) method or Grocott-Gomori methenamine silver (GMS) method. It should be mentioned that there is a risk of

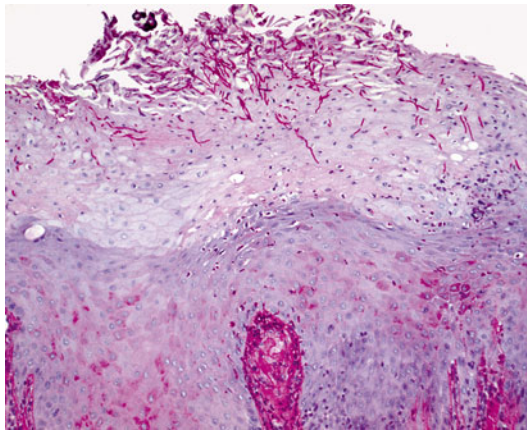

Fig. 8.5 Photomicrograph of a biopsy from a chronic hyperplastic candidiasis. Numerous PAS-positive (*red*) hyphae are seen in the parakeratin layer of the epithelium

false-negative results if only few PAS-stained sections are examined (Roed-Petersen et al. 1970). Hyphae are seen in the parakeratin layer of the epithelium as a superficial infection (Fig. 8.5). Only in severely immunocompromised patients can hyphae be seen in the underlying epithelial layers or in the connective tissue. Typically, the epithelium is hyperplastic and hyperparakeratinized with leukocytes, in particular polymorphonuclear neutrophils, penetrating the epithelium often forming microabscesses in the superficial part of the epithelium in relation to the hyphae. Chronic inflammation is present in the connective tissue underneath the epithelium; however, this is often lacking in severely immunocompromised patients, e.g. patients with HIV infections and AIDS.

8.2.2 Immunological Aspects

A large variety of proinflammatory and immunoregulatory cytokines are generated in the oral mucosa during an infection with *Candida* (Dongari-Bagtzoglou and Fidel 2005). It has been shown that highly invasive strains of *C. albicans* trigger production of proinflammatory cytokines, including IL-1α, IL-6, IL-8 and TNF-α in epithelial cells and IL-6, IL-8, monocyte chemotactic protein (MCP)-1, MCP-2 and granulocyte colony-stimulating factor in endothelial cells (Villar et al. 2005; Whiley et al. 2012).

8.3 Oral Candidiasis in the Medically Compromised Patient

Oral candidiasis is the result of yeast overgrowth and penetration of the epithelial mucosal protective barrier and is evident in patients with various conditions that exhibit immunosuppression including diabetes.

A large number of local and systemic factors and conditions may predispose individuals to oral candidiasis. Factors like treatment with antibiotics, ill-fitting dentures, poor oral hygiene and tobacco smoking can favour growth of yeast cells by disrupting the ecological balance on the mucosal surface (Baboni et al. 2009; Holmstrup and Bessermann 1983; Semlali et al. 2014). Treatment with immunosuppressives, cancer therapy, immune deficiencies, salivary gland hypofunction induced by medication or disease and diabetes can compromise local or general defence mechanisms and thereby lead to oral candidiasis. Often a patient will present several predisposing factors at the same time, and multiple interventions must be initiated including instruction, motivation and follow-up of oral hygiene procedures, diet change, stimulation of functional salivary glands, substitution of xerogenic drugs, diagnosis of oral mucosal diseases, tobacco counselling and smoking cessation and multidisciplinary diagnostic workup of systemic predispositions.

8.3.1 Oral Candidiasis as Adverse Drug Reaction

Systemic antibiotics are the most common cause of acute mucosal candidiasis. Oral exposure to topical glucocorticoid steroids is another common cause of medication-induced oral candidiasis, probably due to alterations of the local mucosal host immunity. Removal of the topical medication with water after exposure tends to prevent recurrence. General immune suppression due to systemic glucocorticoids, cancer chemotherapy and immunomodulators often causes mucosal candidiasis, which usually can be

prevented by prophylactic antifungal treatment. Studies using both culture and pyrosequencing have shown that the oral mycobiota in immunosuppressed solid organ transplant recipients is dominated by *Candida* species (Charlson et al. 2012; Diaz et al. 2013; Dongari-Bagtzoglou et al. 2009). Salivary gland dysfunction resulting in reduced salivary flow rate (salivary hypofunction), compositional changes or a combination of both often has major consequences for the oral microbial balance and local immune defence leading to an increased risk of dental caries and oral candidiasis (Dawes et al. 2015). The main causes of salivary gland hypofunction are intake of medications, systemic diseases like Sjögren's syndrome and head and neck radiotherapy (Villa et al. 2015; Jensen et al. 2010; Pedersen 2014).

A number of studies have shown that intake of xerogenic medication is associated with high rates of *Candida* carriage and oral candidiasis (Almståhl and Wikström 2003, 2005; Kaplan et al. 2008; Pedersen et al. 2015) (for further details, see Chap. 9).

8.3.2 Diabetes Mellitus and Oral Candidiasis

The carriage frequency of *Candida* and the density of candidal colonization as well as the rates of oral *Candida* infections are increased in patients with both type 1 and type 2 diabetes mellitus (DM) (Tapper-Jones et al. 1981; Lamey et al. 1988, 1992; Hill et al. 1989; Vazques and Sobel 1995; Bai et al. 1995; Guggenheimer et al. 2000; Kadir et al. 2002; Jurevic et al 2003; Shenoy et al. 2014). Oral candidiasis is not only more common in patients with DM than in nondiabetics, the infections are also more severe (Guggenheimer et al. 2000). The increased susceptibility to oral candidiasis has been related to poor glycaemic control and hence high concentrations of glucose in the blood and saliva, long disease duration as well as presence of diabetic complications (retinopathy) (Bai et al. 1995; Bartholomew et al. 1987; Dorocka-Bobkowska et al. 1996; Guggenheimer et al. 2000; Kadir et al. 2002; Ueta et al. 1993; Vazques and Sobel 1995). High concentrations of glucose in the blood and saliva may promote growth and enhance adherence of yeasts to epithelial cell surfaces (Samaranayake 1990). Also the impaired functions of polymorphonuclear leukocytes leading to reduced phagocytosis, intracellular killing and chemotaxis may contribute to the increased colonization of *Candida* and increased susceptibility to oral candidiasis (Ueta et al. 1993; Vazques and Sobel 1995). However, other risk factors such as salivary gland hypofunction, low salivary pH and impaired salivary antimicrobial activity, poor oral hygiene, cigarette smoking and denture wearing also have a substantial influence on candidal colonization and various oral manifestations and symptoms of *Candida* infections in both type 1 and 2 DM patients (Budtz-Jørgensen 1990; Banoczy et al. 1987; Guggenheimer et al. 2000; Jurevic et al. 2003; Kadir et al. 2002; Pedersen 2004; Samaranayake 1990; Willis et al. 1999). Inadequately controlled diabetics who wear dentures have a higher oral candida load and higher prevalence of denture stomatitis than nondiabetic denture wearers (Guggenheimer et al. 2000; Vitkov et al. 1999).

C. albicans is the most common species isolated from the oral cavity of diabetics (Dorocka-Bobkowska et al. 1996; Kadir et al. 2002; Samaranayake 1990; Willis et al. 1999), but also *C. dubliniensis*, *C. glabrata* and *C. tropicalis* have been isolated from the oral cavity of patients with diabetes (Jurevic et al. 2003). The significance of species in relation to pathogenesis of fungal infections in diabetics remains to be elucidated.

8.3.3 HIV Infection and Oral Candidiasis

Oral candidiasis is the most common opportunistic infection in HIV-infected patients and in patients with AIDS (Coleman et al. 1993; Shiboski et al. 2015) and one of the earliest indicators of the progression from HIV-seropositive status to AIDS. Oral candidiasis is strongly associated with immune suppression, as measured by $CD4^+$ lymphocyte counts, and also associated with high viral burden and consequently suggested as a clinical marker of plasma

viral load and the progression of HIV disease (Glick et al. 1994; Patton 2000).

The candida carriage in HIV-infected patients is predominated by *C. albicans*, but *C. dubliniensis* and *C. glabrata* have also commonly been isolated from oral lesions in HIV-infected patients (Sullivan et al. 1995; Li et al. 2007). The increased carriage of *C. krusei* has been associated with the widespread use of fluconazole prophylaxis (Samaranayke and Samaranayke 1994). A recent study on the oral mycobiome using pyrosequencing showed a shift in the oral mycobiome in which *Epicoccum* and *Alternaria* were abundantly colonizing HIV-infected patients, but *Candida* being abundant in both HIV patients and healthy subjects (Mukherjee et al. 2014).

The introduction of highly active antiretroviral therapy (HAART) has changed the epidemiology of oral candidiasis. Thus, several studies have reported a decrease in the prevalence and recurrence of oral candidiasis in HIV-infected patients receiving HAART (Greenspan et al. 2004; Jiang et al. 2014; Ramírez-Amador et al. 2007). However, some studies report rare cases of increased prevalence. There is substantial evidence suggesting that onset of oral candidiasis is associated with a progressive reduction in $CD4^+$ lymphocyte count and an increase in viral load in HIV-infected patients receiving HAART (Hodgson et al. 2006; Ramírez-Amador et al. 2007). Oral candidiasis has therefore been suggested as a clinical marker of immune status and a predictor of virologic failure during HAART and hence an indicator of HIV disease progression and a tool for monitoring HIV infection in conjunction with $CD4^+$ lymphocyte counts and plasma viral load in HIV-infected patients receiving HAART (Ramírez-Amador et al. 2007).

8.3.4 Recipients of Organ and Haematopoietic Cell Transplants and Cancer Therapy

Recipients of solid organ transplants and haematopoietic cell transplants and patients receiving cancer therapy have a high risk of developing fungal infections due to immunosuppressive, immunomodulating therapy as well as treatment with antibiotics (Trenschel et al. 2000). Despite antifungal prophylaxis, the increased risk for systemic and oropharyngeal fungal infection is still a matter of concern in these patients, and fungal infections remain a significant cause of morbidity and mortality. The prevalence of oral candidiasis in renal transplant recipients ranges from 9.4 to 46.7 % (Al-Mohaya et al. 2002; de la Rosa-García et al. 2005; Güleç et al. 2003).

C. albicans has been found to be the most prevalent species isolated from the oral cavity of recipients of kidney transplants (da Silva-Rocha et al. 2014). Also in recipients of liver transplants, the *Candida* carriage and prevalence of oral candidiasis are high (40–50 %), and many of these patients also suffer from salivary gland hypofunction (Helenius-Hietala et al. 2014).

The most common forms of oral candidiasis reported in patients receiving cancer therapy are pseudomembranous and erythematous candidiasis (Lalla et al. 2010). There appear to be no relation to *Candida* colonization and the presence or severity of oral mucositis in haemopoietic progenitor cell transplant patients (Epstein et al. 2003; Westbrook et al. 2013), whereas *C. glabrata* has been associated with oral ulcerations in this patient group (Laheij et al. 2012). A recent study indicates that the mycobiome plays a role in the pathogenesis of acute graft-versus-host disease in blood- and marrow-transplanted patients (van der Velden et al. 2013).

Several studies have shown that the oral microbiota is disturbed in patients, who has received radiotherapy in the head and neck region, and in this regard found increased colonization of *Candida* species and higher occurrence of oral candidiasis (Al-Nawas and Grötz 2006; Almståhl and Wikström 2003; Almståhl et al. 2008; Brown et al. 1975; Grötz et al. 2003). *C. albicans* is the cause of the majority of oropharyngeal infections, but *C. glabrata* and *C. tropicalis* are emerging causes of these infections in patients with head and neck cancer. It has been shown that about 50 % of patients with head and neck cancer were colonized with *Candida* species prior to radiotherapy, and after the therapy

the percentage has increased to about 75 % (Lalla et al. 2010). The increased colonization also translated into an increased rate of oral infections. Predisposing factors in patients with head and neck cancer include mucosal injury due to cancer therapy, salivary gland hypofunction, smoking and wearing dentures.

8.4 Chronic Mucocutaneous Candidiasis

Chronic mucocutaneous candidiasis (CMC) is persistent or recurrent widespread superficial *Candida* infection of the oral, oesophageal, digestive, genital, nail mucous membranes and/or skin. Most often *C. albicans* is the infectious *Candida* species. CMC is caused by immune deficiencies involving the mucosal cutaneous immunity, which can have an inherited but most often have sporadic origin. T-cells seem to play an essential role, as several T-cell immune deficiencies, e.g. impaired T-cell function, activation and cytokine signalling, have been associated with CMC (Lanternier et al. 2013). Findings such as reduced proportion of interleukin-17 (IL-17)-secreting T-cells, autoantibodies directed against IL-17 and mutations in IL-17-related genes suggest that impairment of IL-17 immunity plays a significant role in CMC pathogenesis (Puel et al. 2012). CMC is associated with three syndromes including autosomal recessive autoimmune polyendocrinopathy syndrome type 1, hyper IgE syndrome and CARD9 deficiency (Al-Herz et al. 2011). CMC as the principal symptom or CMC in association to other skeletal, endocrine or skin abnormalities initiates in early childhood, and immune dysfunction should be suspected in patients without predisposing risk factors for oral candidiasis.

8.5 Diagnostic Methods

Identification of candidal overgrowth can be established by a variety of methods including culture, cytosmears, biopsy and molecular techniques (Table 8.2).

8.5.1 Culture of Clinical Samples in Order to Quantify and Identify the Candida Load

Different culture media can be used to grow and differentiate *Candida* spp. Some can easily be prepared, e.g. Sabouraud dextrose and Pagano-Levin agar media, and others are commercially available, e.g. CHROMagar™ (CHROMagar, France), chromID® Candida (BioMérieux, USA) and BiGGY Agar (Nickerson Agar) (Sigma-Aldrich®, USA). Up to four different *Candida* spp. can be differentiated by culture of clinical samples. Swaps, imprints, whole saliva and oral rinse can be the source for culture techniques. In immunocompetent patients, there is no universal arbitrary threshold level of colony-forming units (CFU) differentiating between carrier state and candidiasis as individual host and oral environmental factors influence the candida load (Epstein et al. 1980). However, in immunocompromised patients an arbitrary value of >400 CFU has been suggested for antimycotic intervention (Ship et al. 2007). Culture is time consuming and 48 h growth at 37 ° C delays the diagnosis. The composition of the *Candida* spp. in lesional infections may vary from the overall composition in the oral cavity, which make the choice of sampling procedure important (Kragelund et al. 2013).

8.5.2 Smears

Exfoliative cytologic examination is an easy and inexpensive method for detection of candida organisms. The suspected area is vigorously scraped with a wooden spatula and made into a smear on a glass microscope slide, spray fixed with a commercial spay or fixed in 70 % ethanol and stained appropriately, e.g. the PAS or GMS method. An advantage of this method compared to simple culture techniques is that the pathogenic form of the candida organism (hyphae) is easily identified. However, standardized culture of saliva or oral rinse samples is more suitable for identifying candida presence and load in the oral cavity.

Table 8.2 Diagnostic methods, detection techniques and their advantages and disadvantages

Infection	Method	Traditional techniques	Time (hours)	Traditional advantages	Molecular techniques	Time (hours)	Molecular advantage	Disadvantages
Focal mucosal infection	Swab Imprint	CFU	48	Quantification Some species identification	PCR/ MALDI-TOF	24–48 or 10 min.	Species identification	No morphological differentiation Time
	Cytosmear	PAS microscopy	~2	Quantification Morphological differentiation	FISH	~2	Species identification	Expensive
	Cytobrush	DNA			PCR	24–48	Species identification	No morphological differentiation No quantification Time
	Biopsy	PAS microscopy	30	Some quantification Morphological differentiation	FISH	~2	Species identification	Expensive
Generalized oral infection	Whole saliva	CFU	48	Quantification Some species identification	PCR/ MALDI-TOF	24–48 10 min.	Species identification	No morphological differentiation Time
	Oral rinse	CFU	48	Quantification Some species identification	PCR/ MALDI-TOF	24–48 10 min.	Species identification	No morphological differentiation Time

CFU colony-forming unit on culture media, *PAS* Periodic Acid-Schiff, *DNA* deoxyribonucleic acid, *PCR* polymerase chain reaction, *MALDI-TOF* matrix-assisted laser desorption ionization-time of flight, *FISH* fluorescence in situ hybridization

8.5.3 Biopsy

Biopsy is particularly relevant in the case of the hyperplastic candida infections (see above). An appropriate staining method will reveal candida hyphae in the keratin layer of the epithelium. If a suspected lesion does not respond to antifungal therapy, a biopsy may be indicated in order to rule out an underlying disease.

8.5.4 Molecular Techniques for Candida Identification

Molecular techniques make it possible to identify microorganisms based on their genetic or protein uniqueness (Table 8.2). Polymerase chain reaction (PCR) is used to amplify a unique gene sequence of CFU and sample material from cytobrush from lesional foci with candida infections (Kragelund et al. 2013). Pyrosequencing of whole genome sequence or specific gene sequence, e.g. *ERG11* gene coding for the CYP51 the target for azoles, can be performed in order to identify new species or azole resistance (Xie et al. 2014). PCR procedures are time consuming as restriction enzyme analysis or sequencing is necessary for *Candida* identification. Fluorescence in situ hybridization (FISH) can be used on microscope slides or on biopsy material for identification of *Candida* spp. using fluorescence probes for specific DNA or RNA sequences. Commercially available PNA probes (AdvanDx, USA) can differentiate between *C. albicans/parapsilosis*, *C. tropicalis* and *C. glabrata/krusei*.

Identification of microorganisms, e.g. *Candida* spp., using their unique proteomic can be conducted by matrix-assisted laser desorption ionization-time of flight/mass spectrometry (MALDI-TOF/MS). Detection of highly abundant ribosomal proteins of the unidentified microorganism is matched with stored reference MALDI-TOF spectra, and the microorganisms are identified by their typical protein spectrum. It is important that the MALDI-TOF reference database is updated continually because new *Candida* spp. are identified (Criseo et al. 2015). Once the MALDI-TOF equipment has been obtained, it is a cheap and quick technique and is part of routine procedures in many medical microbiological laboratories (Coronado-Castellote and Jimenez-Soriano 2013).

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9 Association Between Oral Infections and Salivary Gland Hypofunction

Siri Beier Jensen and Anne Marie Lynge Pedersen

Abstract

Saliva plays an important role in the maintenance of oral health and regulation of the oral microbiota. Saliva lubricates the oral hard and soft tissues, dilutes food detritus and bacteria and enhances the clearance of microorganisms and dietary carbohydrates from the oral cavity. Saliva also provides antimicrobial activity via numerous proteins and peptides including lactoferrin, lactoperoxidase, lysozyme, statherin and histatins. This chapter focuses on the oral microbiota in patients suffering from salivary gland hypofunction due to Sjögren's syndrome, radiotherapy of tumours in the head and neck region, cancer chemotherapy and intake of medications. Despite the different causes of salivary gland hypofunction, these patient groups show some similarities regarding the composition of the oral microbiota with increased colonisation of oral pathogens associated with dental caries (*Streptococcus mutans* and *Lactobacillus* species) and oral mucosal infections, especially *Candida albicans*.

9.1 Introduction

Saliva plays an essential role in the maintenance of tooth integrity and protection against dental caries by neutralising acids from food and bacteria via salivary buffering systems, contributing to formation of the dental pellicle, diluting food detritus and bacteria and mechanical cleansing of the oral cavity. Furthermore, salivary proteins such as statherin and proline-rich proteins keep saliva supersaturated with respect to calcium phosphate salts thereby preventing demineralisation. Similarly saliva and its components maintain mucosal integrity by constantly covering and lubricating the oral soft tissues and thereby preventing injuries as well as adhesion and proliferation of microorganisms. In addition, saliva provides antimicrobial activity via a large variety of proteins and peptides including mucins, lysozyme, lactoferrin, histatins, defensins and

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antibodies (secretory IgA) and thereby inhibits bacterial and fungal colonisation and infections (Dawes et al. 2015; Lagerlöf and Oliveby 1994; Lenander-Lumikari and Loimaranta 2000).

The composition of saliva is dependent on the rate by which the saliva is produced, the type of gland, from which the saliva is secreted and the nature and duration of the stimuli applied to activate the secretion reflexes (Pedersen et al. 2002a, b). The composition of salivary antimicrobial proteins may therefore vary from one oral site to another in an individual, and different oral sites can harbour different microbiota depending on the local morphology, growth conditions and the local immune defence. Other factors such as oral hygiene, diet, dental restorations, systemic diseases, medication intake and various lifestyle factors also affect the local oral microbiota (for further details see Chap. 2).

Under normal conditions, the unstimulated whole saliva flow rate is on average 0.3–0.4 ml/min, while chewing-stimulated whole saliva flow rate is about 1.5–2.0 ml/min (Humphrey and Williamson 2001; Pedersen et al. 2002a, b). An unstimulated whole saliva flow rate ≤0.1 ml/min and chewing-stimulated whole saliva flow rate ≤0.5–0.7 ml/min are designated hyposalivation (Heintze et al. 1983; Pedersen et al. 2002a, b). Xerostomia usually occurs when the unstimulated secretion rate has decreased to approximately 50 % of its normal value in any given individual, indicating that more than one major salivary gland must be affected (Dawes 1987).

The importance of saliva in the maintenance of a natural balance between the host and the oral microbiota becomes evident when the saliva flow rate is reduced. The most common causes of salivary gland hypofunction include intake of medications, polypharmacy, systemic diseases such as Sjögren's syndrome and cancer therapy including chemo- and radiotherapy. Regardless of the aetiology of salivary gland hypofunction, a shift in the oral ecology appears to occur already at unstimulated whole saliva flow rates below 0.20 ml/min with a shift towards a more aciduric and acidophilic oral microbiota leading to an increased risk of dental caries and oral candidiasis (Navazesh et al. 1995; Bardow et al. 2001). This chapter reviews results from studies of the oral microbiota both in rinsing samples and in samples collected from specific sites in patients with chronically or temporary salivary gland hypofunction due to Sjögren's syndrome, cancer therapy (chemo- and/or radiotherapy) and intake of medications.

9.2 Oral Microbiota in Sjögren's Syndrome

Sjögren's syndrome (SS) is a chronic, systemic autoimmune inflammatory disorder that affects the exocrine glands, particularly the salivary and lacrimal glands. The most prominent disease manifestations include hyposalivation and keratoconjunctivitis sicca resulting in symptoms of oral and ocular dryness. The aetiology remains unknown, but most likely includes an interaction between immunological, genetic, hormonal and environmental factors. The median age of presentation is around 50 years and it mainly affects women. SS is classified into two forms: primary SS (pSS) and secondary SS (sSS). The latter defines the disease entity in the presence of another chronic inflammatory connective tissue disease, most commonly rheumatoid arthritis or systemic lupus erythematosus (Pedersen and Nauntofte 2005). Diagnosis is often delayed which reflects the fact that the onset is often insidious and patients present various and unspecific symptoms like xerostomia, fatigue, myalgia and arthralgia. Hyposalivation may therefore precede the diagnosis for several years increasing the risk of dental caries and recurrent oral candidiasis. Before diagnosis and hence awareness of risk of oral diseases, the patients may have a frequent intake of easily fermentable carbohydrates like candies and soft drinks in order to alleviate the symptoms of dry mouth (Brunström 2002; Cermak et al. 2003) and an inadequate oral hygiene which further favour the growth of *Streptococcus mutans* and *Lactobacillus* and *Candida* species.

9.2.1 Dental Caries

Both the quantity and quality of saliva are affected in patients with pSS (Kalk et al. 2001; Pedersen

et al. 2005; Thorn et al. 1989). The number of decayed, missed and filled teeth is high (Baudet-Pommel et al. 1994; Christensen et al. 2001; Pedersen et al. 1999a, 2005) and found inversely correlated to salivary flow rates and especially the unstimulated whole saliva flow rate (Pedersen et al. 1999b, 2005). The reduced saliva secretion results in reduced bicarbonate concentration, pH and buffer capacity (Bardow et al. 2001; Pedersen et al. 2005). The clearance of microorganisms and dietary sugars is also impaired thereby promoting an environment dominated by aciduric and acidogenic species and prolonged exposure of dietary sugars and acids to the teeth. Kolavic et al. (1997) found higher counts of *Streptococcus mutans* and lactobacilli in caries-inactive patients with SS having stimulated parotid flow rates <0.25 ml/min than in subjects with higher parotid flow rates. The counts and numbers of *S. mutans* and lactobacilli counts have been found inversely correlated to stimulated whole saliva flow rates (Lundström and Lindström 1995). Almståhl et al. (1999) also showed that patients with pSS harboured higher numbers of both *S. mutans* and *Lactobacillus* species and sSS higher numbers of *Lactobacillus* species than healthy subjects. In patients with pSS, the shift in the oral microbiota appears to occur despite a good oral hygiene, as they had higher levels of *S. mutans* than patients who had received radiotherapy in the head and neck region and patients who took neuroleptics (Almståhl et al. 1999). The high number of microbial retention sites generated by dental restorations such as fillings, crowns and bridges found in patients with pSS may also contribute to the shift in oral bacteria (Almståhl et al. 1999). Leung et al. (2007) found higher levels of lactobacilli in saliva, especially *L. acidophilus*, *L. fermentum* and *L. minitus*, and in supragingival plaque from patients with SS than in subjects with normal salivary secretion, but no differences in the numbers of *S. mutans* or anaerobic gram-negative rods.

9.2.2 Oral Candidiasis

Recurrent oral candidiasis is prevalent among patients with SS, and the most common clinical presentation of *Candida albicans* colonisation is erythematous candidiasis and angular cheilitis (Hernandez and Daniels 1989; Lundström and Lindström 1995; Pedersen et al. 1999b; Soto-Rojas et al. 1998; Tapper-Jones et al. 1980). *C. albicans* is the most frequently isolated (66–72 %) species in patients with SS. It may occur alone or mixed with other *Candida* species such as *C. tropicalis*, C. *pseudotropicalis*, *C. parapsilosis*, *C. kefyr* and *C. glabrata* (Kindelan et al. 1998; Soto-Rojas et al. 1998). The prevalence of *Candida* as well as the numbers of colony-forming units (CFU) per ml (CFU/ml) not only varies between studies but also between the pSS and sSS patients (Table 9.1) reflecting differences in the patient groups regarding dental status, oral hygiene habits, comorbidity, medication intake and/or immune response. Results of saliva cultures and oral rinses correspond well to the occurrence of signs and symptoms of oral candidiasis (Abraham et al. 1998; Kindelan et al. 1998; Soto-Rojas et al. 1998). Candidal colonisation and oral candidiasis tend to be more prevalent in patients with sSS (Soto-Rojas et al. 1998). Almståhl et al. (2001) showed that patients with pSS had significantly higher levels of *C. albicans* in rinsing samples than subjects with hyposalivation of unknown aetiology. SS patients with immeasurable unstimulated whole saliva flow rates have the highest levels of *C. albicans* in oral rinses (Almståhl et al. 1999).

The microbial samples proving the presence of *Candida* species on the oral mucosa have usually been obtained by smears or culture swabs taken from the dorsum of the tongue, the buccal or palatal mucosa, the right tonsillar area and/or the fitting surface of the denture (Almståhl and Wikström 1999; Leung et al. 2008; MacFarlane and Mason 1974; MacFarlane 1984; Pedersen et al. 2002a, b; Rhodus et al. 1997; Soto-Rojas et al. 1998; Tapper-Jones et al. 1980). Results revealed that patients with SS have a significant higher mucosal colonisation of *C. albicans* than healthy subjects (Leung et al. 2008; MacFarlane 1984; Radfar et al. 2003; Rhodus et al. 1997; Soto-Rojas et al. 1998; Tapper-Jones et al. 1980; Yan et al. 2011) and pSS compared with patients with oral lichen planus (Pedersen et al. 2002a, b). Patients with sSS harboured higher numbers of

Table 9.1 Frequency of *Candida* species determined semiquantitatively and the numbers of colony-forming units per ml (CFU/ml) in patients with primary Sjögren's syndrome (pSS) and secondary Sjögren's syndrome (sSS)

Microbiological tests	pSS	sSS	References
Tongue smear	33 %	76 %	Sota-Rojas et al. (1998)
Tongue swab culture	52 %	76 %	Sota-Rojas et al. (1998)
Saliva culture/oral rinse	76 %	79 %	Sota-Rojas et al. (1998)
	81 %	67 %	Kindelan et al. (1998)
	65 %	60 %	Almståhl et al. (1999)
	72 %	48.1 %	Leung et al. (2007)[a]
Supragingival plaque	84 %	55.6 %	Leung et al. (2007)[a]
Numbers of CFU/ml			
Tongue/palate swab culture	3.1×10^6 (mean)	1.2×10^5	Rhodus et al. (1997)
Saliva culture	419/µl (mean)	739/µl	Sota-Rojas et al. (1998)
	$>10^4$ (35.7 %)	$>10^4$ (39.1 %)	Ergun et al. (2010)
Oral rinse	2100 (median)	1710	Kindelan et al. (1998)[a]
	380 (median)	500	Almståhl et al. (1999)[b]
	1025.5 (mean)	155	Leung et al. (2007)[a]
Supragingival plaque	1.8×10^6/g (mean)	0.4×10^6	Leung et al. (2007)[a]

[a]The study included denture wearers
[b]The study only included dentate subjects

C. albicans (CFU 3.1×10^6) than patients with pSS (CFU 1.2×10^5), which was attributed to the presence of an additional inflammatory disease in sSS as well as to low saliva flow rates (Rhodus et al. 1997). The presence and density of *C. albicans* were also inversely correlated to saliva flow rates (Hernandez and Daniels 1989; Radfar et al. 2003; Rhodus et al. 1997; Tapper-Jones et al. 1980). Hernandez and Daniels (1989) found that patients with SS and with chronic erythematous candidiasis were older and had long duration of oral symptoms, more inflammation in their labial salivary glands and lower stimulated parotid flow rates than SS patients without this oral lesion. In contradiction to oral rinses, not all mucosal cultures correspond to the clinical signs and symptoms. Thus, MacFarlane (1984) found that 73 % of the patients with pSS had clinical signs of oral candidiasis, although cultures obtained from the dorsal part of the tongue only were positive in 52 % of the cases. This difference between clinical signs and results of *Candida* cultures may reflect difficulties in obtaining representative material from the dry mucosa (Lundström and Lindström 1995; Soto-Rojas et al. 1998). Furthermore, patients with *Candida* infection do not necessarily exhibit oral lesions which can be attributed to an asymptomatic carrier status or early candidiasis without clinical apparent lesions or a less virulent strain of *Candida*. Regarding site specificity, it is noteworthy that *C. albicans* was found twice as frequent in the supragingival plaque than on the tongue in patients with pSS, but could not be detected in the gingival crevicular region using the paper point technique (Almståhl et al. 2001b).

9.2.3 Other Microorganisms

A sparse number of studies have examined the bacteria in oral mucosal cultures of patients with SS. MacFarlane and Mason (1974) found significantly higher numbers of *Staphylococcus aureus* and coliform bacilli in SS patients without clinical signs of inflammation than in healthy subjects. *Veillonella* species, *Neisseria pharyngis*, *Micrococcus mucilaginosus*, *S. salivarius* and *S. aureus* have also been isolated in higher numbers from the tongue, palate, throat and dentures of patients than in healthy subjects (MacFarlane 1984). However, Almståhl and Wikström (1999) found no differences in the numbers of *S. aureus* and enterics between patients with pSS and healthy subjects, and the numbers of *S. salivarius*, *Neisseria pharyngis* and *Veillonella* species were

lower in pSS patients. Regarding site specificity, a higher density of streptococci, *S. salivarius*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Prevotella nigrescens* and *S. aureus* has been demonstrated on the dorsal part of the tongue than on the buccal mucosa and in the vestibulum (Almståhl et al. 2001b). Species usually associated with gingivitis such as *F. nucleatum*, *P. intermedia* and *P. nigrescens* were found in slightly lower levels in the gingival crevice region of the patients with pSS, but in higher levels of the sSS patients, than in control subjects (Almståhl and Wikström 1999). In this regard, it is noteworthy that the susceptibility to gingivitis and periodontitis has not been found increased in patients with pSS compared to healthy controls (Boutsi et al. 2000; Kuru et al. 2002; Pedersen et al. 1999b; Schiødt et al. 2001; Tseng et al. 1990), which is supported by the rare detection of *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* in the gingival crevice region of patients with pSS (Almståhl et al. 2001b). However, increased levels of antibodies to *A. actinomycetemcomitans* and *Porphyromonas gingivalis*, but not to *P. intermedia*, in addition to an increased incidence of periodontal disease have been reported in patients with SS (Çelenligil et al 1998; Ergun et al. 2010), but they did not discriminate between pSS and sSS, and it is likely that patients with sSS display an increased risk of periodontal diseases due to its concomitant presence with rheumatoid arthritis (for further details on rheumatoid arthritis and periodontitis, see Chap. 4).

9.3 Oral Microbiota in Patients Receiving Chemotherapy

During cancer chemotherapy, there is a significantly increased risk of oral infections due to the immunosuppressive effect and the direct cytotoxic effect of the drugs on oral epithelial barrier function. Studies have shown that chemotherapy may induce temporary salivary gland hypofunction, although there is some controversy whether it is caused by the chemotherapy per se or by other factors, e.g. concomitant intake of xerogenic medication like antiemetics (Jensen et al. 2010). Salivary secretion may even be lower in individuals with cancer prior to the initiation of treatment (Harrison et al. 1998; Napeñas et al. 2013). Chemotherapy can induce compositional changes in saliva. Decreased saliva flow rates combined with the finding of slightly increased salivary sodium and chloride concentrations as well as decreased inorganic phosphate concentration suggest that salivary gland acinar secretion and duct modification mechanisms are impaired by cancer chemotherapy (Jensen et al. 2008a). The concentration and output of secretory IgA has been found to decrease both during and following chemotherapy (Harrison et al. 1998; Jensen et al. 2008a; Laine et al. 1992; Main et al. 1984; Meurman et al. 1997a) and the concentration of lysozyme to decrease after chemotherapy (Meurman et al. 1997a). The salivary peroxidase system is impaired during chemotherapy due to a lower concentration of thiocyanate and its oxidised form hypothiocyanite with antibacterial properties (Mansson-Rahemtulla et al. 1992). Findings on saliva pH and buffer capacity are inconsistent and have been shown to decrease, be unchanged or with regard to buffer capacity even be increased in response to cancer chemotherapy (Avsar et al. 2007; Jensen et al. 2008a; Nemeth et al. 2014; Pajari et al. 1989; Schum et al. 1979). Thus, salivary gland hypofunction, changed composition and reduced output of antimicrobial substances may impair the oral host defence against microorganisms, thus individuals in cancer chemotherapy may be more susceptible to oral infections.

9.3.1 Dental Caries

Increased amount of dental bacterial plaque, gingival inflammation and increased salivary counts of caries-related bacteria, mutans streptococci and lactobacilli have been found during and after chemotherapy (Avsar et al. 2007; Jensen et al. 2008b). However, other studies have shown that the salivary concentrations of *S. mutans* and lactobacilli may decrease during chemotherapy in spite of salivary gland hypofunction. This could be ascribed to the concomitant use of antibiotics, antifungals

and/or chlorhexidine mouth rinses during chemotherapy as well as cytotoxic effect of the chemotherapy itself (Meurman et al 1997b; O'Sullivan et al. 1993). *S. mutans* has been found to be sensitive to daunorubicin, a cytotoxic antibiotic used in chemotherapeutic regimens (O'Sullivan et al. 1993). Along this line, a study showed that salivary *S. mutans* counts decreased, whereas lactobacilli counts increased during chemotherapy (Meurman et al. 1997b). Other studies have not revealed any changes in the composition of the oral microbiota (Bergmann 1991; O'Sullivan et al. 1993; Wahlin and Holm 1988), but an initial doubling of the concentration of microorganisms concomitant with a transient decrease of stimulated whole saliva flow rate during chemotherapy (Bergmann 1991). A study of the supra- and subgingival dental plaque in adult acute leukaemia patients during chemotherapy found that the percentage of total viable counts of *S. mutans* in supragingival dental plaque increased and the percentage in subgingival dental plaque decreased (Reynolds et al. 1989). However, the percentage of viridans streptococci (*S. mutans* not specified) has also been shown to be lower in the supragingival dental plaque of children with acute leukaemia during chemotherapy than in healthy individuals (Sixou et al. 1998).

As chemotherapy is a time-limited treatment and caries is a process that progresses relatively slowly, it may be debatable whether it is possible to assess an increased progression rate of caries during chemotherapy. One study found that 5 years after chemotherapy, salivary counts of *S. mutans* and lactobacilli were on the same low levels as baseline values before chemotherapy (Meurman et al. 1997b). Another study found no significant correlation between salivary immunoglobulin levels in stimulated whole saliva and *S. mutans* or *Lactobacillus* counts in long-term (6 months to 10 years) event-free paediatric patients treated for childhood malignancies by chemotherapy (Dens et al. 1995). The salivary immunoglobulin level was within normal limits, but there was a negative correlation between secretory IgA concentration and caries prevalence (DMFT/dmft), although only significant in some age groups. In bone marrow transplant patients, a significant decrease in stimulated whole saliva flow rate, lower buffer capacity and a change in the oral microbiota towards higher salivary counts of *S. mutans* and *Lactobacillus* have been observed during and after chemotherapy and transplantation (Dahllof et al. 1997; Dens et al. 1996). However, stimulated saliva flow rates reached normal values 1 year after cancer treatment, and no significant differences in caries prevalence were found between bone marrow-transplanted children receiving chemotherapy and healthy children 4 years following treatment, but all participants also underwent preventive dental care (Dahllof et al. 1997). In other paediatric populations, significantly more caries, poor oral hygiene and significantly lower stimulated whole saliva flow rate have been found following childhood chemotherapy (Alberth et al. 2004; Avsar et al. 2007; Nemeth et al. 2014; Pajari et al. 1995).

9.3.2 Oral Candidiasis during and After Chemotherapy

The oral yeast counts and especially the prevalence of *Candida* species may increase significantly from a prevalence of about 50 % in the normal population to 73 % in cancer patients during chemotherapy, and the weighted prevalence of clinical oral fungal infection has been estimated to be 38 % (Lalla et al. 2010). *C. albicans* is the predominant yeast in the oral microbiota during chemotherapy and accounts for up to 88 % of the salivary yeasts (Samaranayake et al. 1984). Other potential virulent *Candida* species may colonise the oral cavity during cancer treatment with weighted prevalence of 16.6 % for *Candida tropicalis*, 5.5 % for *Candida glabrata* and 3 % for *Candida krusei* (data pooled for chemotherapy and radiation therapy) (Lalla et al. 2010).

Clinical candidiasis and angular cheilitis have been found to correlate to higher oral yeast counts and low saliva flow rates (Wahlin and Holm 1988; Wahlin 1991). A follow-up study found that salivary yeast counts remained high in spite of normal saliva flow rates 5 years after chemotherapy for lymphoma (Meurman et al. 1997b). The salivary concentrations of

s-IgA, IgG, IgM and lysozyme in stimulated whole saliva were concomitantly found to be significantly decreased as compared to baseline values (Meurman et al. 1997a). These findings suggest that the disease itself or the chemotherapy may affect the body defences against *Candida* in the long term.

9.4 Oral Microbiota in Patients Receiving Radiation Therapy

Radiotherapy (RT) of tumours in the head and neck region often includes the major and minor salivary glands in the radiation field depending on the anatomical location and the extension of the tumour. RT can cause severe salivary gland hypofunction (Jensen et al. 2003, 2010). The severity of salivary gland hypofunction depends on the volume of salivary gland tissue included in the radiation field and on the total radiation dose (Vissink et al. 2010). RT targets cells with a rapid mitotic turnover like tumour cells and damages the DNA thereby leading to cell death. Acinar salivary gland cells are radiosensitive in spite of their slow mitotic turnover (Berthrong 1986), and the serous cells appear to be more sensitive to radiation than the mucous ones (Kashima et al. 1965). Radiation damage to the salivary glands may be seen as early as 1 week after initiation of RT (Dreizen et al. 1977) and results in both acute and long-term effects characterised by reduced saliva flow rates, high saliva viscosity and changes of saliva composition. During RT, saliva flow rates decrease and may even reach immeasurable levels (Vissink et al. 2010). With decreasing flow rates, the salivary pH drops and the buffer capacity decreases both during and after RT (Jensen et al. 2003; Valdez et al. 1993). RT also affects the salivary antimicrobial components. The salivary concentrations of IgA and IgG, lactoferrin, lysozyme and peroxidase have been shown to increase during RT due to acute tissue destruction, but after RT they decrease due to reduced functioning of the glands (Brown et al. 1976; Jensen et al. 2003; Makkonen et al. 1986). In the long term, recovery of salivary gland function is dependent on the total radiation dose that the tissue has received. Thus, saliva flow rates may remain severely decreased, and the compositional changes may persist in response to the decrease in saliva flow rates (Jensen et al. 2003, 2010). The standard therapeutic radiation dose for head and neck carcinoma amounts to a total dose of 60–70 Gy. The major salivary glands may have the potential to gradually recover within 1–2 years if gland-sparing radiation regimens have been applied, for example, intensity-modulated radiation therapy, and if it has been achievable to keep the radiation dose to the gland tissue below thresholds of ~26 Gy to the parotid gland and ~39 Gy to the submandibular gland (Murdoch-Kinch et al. 2008; Vissink et al. 2010). A compensatory increase in saliva flow rate from salivary glands not included in the radiation field may be seen (Eisbruch et al. 2001).

9.4.1 Dental Caries

Irradiation-induced salivary gland hypofunction is associated with a shift of the normal oral microbiota increasing the risk to develop rampant dental caries (Vissink et al. 2003). Higher levels of *S. mutans* and *Lactobacillus* species are often observed in the oral cavity during and after RT compared to preradiation levels (Brown et al. 1975, 1978; Keene and Fleming 1987; Llory et al. 1972; Schwarz et al. 1999; Vuotila et al. 2002). Oral colonisation with *S. mutans* has been found lower and stimulated saliva flow rates higher at the end of treatment in patients receiving unilateral RT as compared to bilaterally irradiated patients (Beer et al. 2002). In oral rinses, it has been demonstrated that the predominant acid-producing species of the oral microbiota may change from *S. sanguis*, *S. mitis* and *S. salivarius* before RT to *S. mitis*, *S. salivarius* and lactobacilli with a concomitant decrease in saliva flow rates after RT (Tong et al. 2003). The acid-sensitive *S. sanguis* appears to be inhibited by the more acidic oral environment after RT (Tong et al. 2003). The decrease in the presence of *S. sanguis* after RT has been shown in other studies (Brown et al. 1975, 1978). Vuotila et al. (2002)

found unchanged levels of *S. mutans* after RT as compared to preradiation levels. Interestingly, a study found no difference in the microbial diversity, composition or number of colony-forming bacterial units (mutans streptococci and lactobacilli), nor did they find a difference in the stimulated whole saliva flow rate, saliva pH and buffering capacity when comparing caries-free and caries-active irradiated nasopharyngeal patients (Zhang et al. 2015).

RT patients suffering from impaired saliva secretion are to be considered a high-risk group regarding dental caries as the oral environment favours acidogenic and acidophilic species.

9.4.2 Oral Candidiasis

The number of yeasts increases in the oral cavity of cancer patients with hyposalivation due to RT, and oropharyngeal candidiasis is a frequent complication during and after RT for head and neck cancer (Lalla et al. 2010). The weighted prevalence of oral candidiasis during head and neck RT has been estimated to be 37 % (Lalla et al. 2010). The increase in the oral yeast colonisation is observed during RT, and the colonisation level remains elevated after RT. The weighted prevalence of oral fungal colonisation during radiation therapy has been estimated to be 75 % (Lalla et al. 2010). *C. albicans* is the predominant yeast species associated with oral candidiasis in RT patients (Redding et al. 1999; Thaweboon et al. 2008). However, other *Candida* species are frequently isolated from the oral cavity in RT and may cause oral candidiasis, e.g. *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei* (Bulacio et al. 2012; de Freitas et al. 2013; Lalla et al. 2010; Schelenz et al. 2011). It has been shown that the increase in *C. albicans* in oral rinses is positively related to the radiation dose and the volume of parotid gland tissue included in the radiation field (Rossie et al. 1987). A direct correlation between the increase in *C. albicans* in saliva and reduced saliva flow rates during RT has also been shown (Epstein et al. 1998; Karbach et al. 2012). The increased colonisation of oral yeasts in RT patients in combination with salivary gland hypofunction emphasises the importance of optimal oral hygiene in RT patients due to the risk of developing oropharyngeal candidiasis (Lalla et al. 2010).

9.5 Medication-Induced Salivary Gland Dysfunction and Oral Microbiota

The most common cause of salivary gland dysfunction is the intake of prescribed medications (Handelman et al. 1986; Närhi et al. 1992; Smidt et al. 2010, 2011; Villa et al. 2015; Österberg et al. 1984). Xerostomia is a common complaint, especially in elderly people above the age of 65 years, and is often reported as an adverse effect of medications. The prevalence is ranging from 11 to 72 % (Smidt et al. 2011; Desoutter et al. 2012). Xerostomia is defined as the subjective feeling of oral dryness (Fox et al. 1987; Shetty et al. 2012), and salivary gland dysfunction denotes changes in the quantity and/or quality of saliva (Villa et al. 2015). More than 75 % of adults aged 65 and older take at least one prescription medication (Chrischilles et al. 1992; Smidt et al. 2010). Xerostomia has been associated with 80 % of the most commonly prescribed medications, and several of them have adverse effects directly on the mechanisms responsible for saliva secretion (Smith and Burtner 1994; Sreebny 2010; Smidt et al. 2011; Villa et al. 2015; Aliko et al. 2015). Regardless of the type of medication, saliva flow rates have been shown to decrease as the number of medications increases also known as polypharmacy (Thorselius et al. 1988; Närhi et al. 1992; Smidt et al. 2010). Xerostomia is not only associated with a decrease in the salivary flow rate, it may also be attributed to a change in quality of the saliva (Crogan 2011; Ekström et al. 2012; Tabak 1995). The duration of medication intake also affects the saliva flow rates and the prevalence of xerostomia (Navazesh et al. 1996; Thomson et al. 2006). Navazesh et al. (1996) found that unstimulated and stimulated whole saliva flow rates were significantly lower in adults who had been taking medication for more than 2 years as compared to those who had been taking medication for less than 2 years.

The mechanisms by which medications can influence salivary secretion and cause salivary

gland dysfunction are complex, and this is also reflected in the variation and severity of oral complications. It is believed that medications can interact with the salivary secretory reflex at several sites (Ekström et al. 2012; Proctor 2015; Sreebny 2010; Villa et al. 2015). Thus some medications act at the level of the central nervous system such as antidepressants (both tricyclic and non-tricyclic), opioids, sedatives, anxiolytics and decongestants (pseudoephedrine). Also antihypertensives acting on central alpha-2 adrenergic receptors (e.g. clonidine) have been shown to reduce salivary secretion in humans (Proctor 2015; Sreebny 2010). Other medications like anticholinergics for overactive bladder, antiemetics, tricyclic antidepressants, serotonin reuptake inhibitors, certain neuroleptics, antihistamines and antihypertensives (alpha-1 adrenergic and beta-adrenergic blocking agents) are acting at the peripheral level of the neuro-glandular junction interfering with cholinergic muscarinic (M3), adrenergic, peptidergic and/or histaminergic receptor systems (for reviews Proctor 2015; Sreebny 2010). Antidepressants, i.e. inhibitors of serotonin and noradrenaline transporters responsible for reuptake, appear to cause salivary gland dysfunction through activation of alpha-2 adrenergic receptors by elevating endogenous levels of noradrenaline. Both centrally and peripherally acting medications include tricyclic antidepressants, the serotonin reuptake inhibitors, some neuroleptics and antihistamines (Clemmesen 1988; Del Vigna de Almeida et al. 2008; Hunter and Wilson 1995; Proctor 2015; Sreebny 2010). Furthermore, some medications like diuretics indirectly influence the salivary secretion by affecting the electrolytes and water homeostasis (Nederfors et al. 1989). Finally, a large number of factors influence the effect of medications on salivary secretion, such as the dose and the absorption and excretion rates of the drug as well as drug interactions (Del Vigna de Almeida et al. 2008).

9.5.1 Dental Caries

A relatively limited number of studies have investigated the association between medication-induced salivary gland dysfunction and changes in the oral microbiota. A cross-sectional study demonstrated that root caries was more prevalent in those taking antihypertensives than in control subjects (Streckfus et al. 1990). Also the whole saliva flow rates have been found lower and the levels of mutans streptococci and lactobacilli higher in patients taking antihypertensives compared to control subjects (Nonzee et al. 2012). A study on 848 community-dwelling elderly people in South Australia found only a moderate association between medication intake and root caries experience (Thomson et al. 1995). However, a more detailed analysis on the various drugs revealed that patients who took antidepressants and antiulcer agents had a significantly higher root caries index and a 5-year follow-up study including 528 community-dwelling elderly South Australians did not reveal a strong association between intake of medication and caries, apart from intake of antiasthmatics. Along this line, Ryberg et al. (1991) found that long-term treatment with β2-adrenoceptor agonists in patients with asthma was associated with an impaired saliva secretion, which was followed by an increased incidence of dental caries and higher number of DMS (Decayed-Missing-Surfaces) after 4 years of follow-up. These findings were supported by Alaki et al. (2013), who also found higher levels of mutans streptococci and lactobacilli in asthmatic patients taking antiasthmatics more than three times a day compared with other asthmatic patients. A recent study showed that patients on diuretics had a higher prevalence of xerostomia, periodontitis, dental caries and mucosal lesions than control subjects (Prasanthi et al. 2014). A study by Rindal et al. (2005) demonstrated that patients taking antidepressants had a higher number of dental restorations (a proxy for dental caries) than the non-medicated ones. Bardow et al. (2001) showed that patients with a daily intake of xerogenic medications had low unstimulated and stimulated whole saliva flow rates and decreased salivary outputs of bicarbonate, calcium, phosphate and protein and higher levels of *Lactobacillus* species. These results were substantiated by Almståhl et al. (2003) who found higher numbers of lactobacilli in oral rinses from patients with medication-induced hyposalivation

than in control subjects, but lower levels than in patients with pSS and patients with RT-induced hyposalivation which was attributed to much lower saliva flow rate in the two latter groups. It has also been shown that patients with medication-induced hyposalivation had a supragingival plaque comprising high levels of mutans streptococci and lactobacilli and hence an increased risk of developing caries (Almståhl and Wikström 2005). An additional number of studies have reported that the numbers of lactobacilli are higher in medicated patients compared to non-medicated and also associated with low saliva flow rates (Fure 2003; Närhi et al. 1994; Parvinen et al. 1984).

Moreover, several liquid formulations of medications have a high sugar content that may influence the oral microbiota and hence increasing the risk of medication-induced caries (Donaldson et al. 2015). Beighton et al. (1991) showed that the salivary level of mutans streptococci, lactobacilli and yeasts in elderly patients treated with sucrose-containing medication was significantly higher than in patients taking non-sucrose-containing medication.

9.5.2 Oral Candidiasis

The number of studies investigating the association between medication-induced salivary gland dysfunction and oral candidiasis is sparse. It has been shown that medicated elderly people have a higher *Candida* load and also higher frequency of oral candidiasis and lower saliva flow rates than the non-medicated ones (Pedersen et al. 2015). A higher frequency of *Candida* isolation and palatal inflammation has also been found in patients treated with psychotropic agents, and who were wearing complete upper dentures, than in control subjects (Lucas 1993). However, other risk factors were more common among the psychiatric patients including cigarette smoking, sugar consumption and a poor denture hygiene. A recent study reported that intake of anxiolytics, and low salivary flow rates, were associated with higher levels of *Candida* in patients with oral lichen planus (Bokor-Bratic et al. 2013). Janket et al. (2007) showed that intake of xerogenic medication was significantly associated with a high oral mucosal inflammation score. Furthermore, in patients taking antihypertensives, the mean levels of *Candida* species were higher than in the control subjects (Nonzee et al. 2012). Other studies have found higher *Candida* levels in patients with medication-induced hyposalivation and in medicated men (Almståhl and Wikström 2005; Parvinen et al. 1984), and Kreher et al. (1991) showed that *C. glabrata* was the most frequent yeast strain in the oral cavity of medicated patients.

9.5.3 Periodontal Disease

One study has found that periodontal disease (assessed by Russell's periodontal index and plaque index) was more prevalent in patients taking diuretics and who also had low whole saliva flow rates (Nonzee et al. 2012). The authors suggest that the high prevalence of periodontitis could be due to decreased cleansing activity and reduced microbial activity by saliva. However, apparently there is no substantial evidence suggesting that medication-induced salivary gland dysfunction is associated with an increased risk of periodontal disease (Aliko et al. 2015). Periopathogens such as *P. gingivalis* and *A. actinomycetemcomitans* are rarely detected in supragingival plaque samples from patients with medication-induced salivary gland hypofunction or in patients with hyposalivation due to other causes (Almståhl and Wikström 2005).

Conclusions

Patients with SS often have a severe and permanent reduction in their salivary gland function leading to reduction in the salivary pH and decreased clearance of microorganisms, dietary sugars and acids in the oral cavity. This leads to a shift in the oral microbiota including colonisation of more acidophilic species such as *S. mutans*, lactobacilli and *C. albicans* and consequently an increased risk of dental caries and oral candidiasis.

During cancer chemotherapy, there is a significantly increased risk of oral infections due

to the immunosuppressive effect and the direct cytotoxic effect of the drugs on oral epithelial barrier function. Moreover, chemotherapy may also induce temporary salivary gland hypofunction, although there is some controversy whether it is caused by the chemotherapy per se or by other factors, e.g. concomitant intake of xerogenic medication like antiemetics. Studies have shown that the output of salivary antimicrobials may decrease during chemotherapy and thus increase the risk of oral infections. Changes in the oral microbiota may not be attributable to the chemotherapy alone, but also occur due to concomitant medication, antimicrobial treatment, the underlying cancer disease and duration of hospitalisation.

RT in the head and neck region can result in a severe and permanent salivary gland hypofunction. The severity depends on the volume of salivary gland tissue included in the radiation field and on the total radiation dose. In cancer patients with RT-induced salivary gland hypofunction, the clearance of microorganisms decreases. Changes in the microbiota include increased colonisation of *Lactobacillus* species, *S. mutans* and *Candida* species, and these patients have a significant increased risk of especially dental caries and oral candidiasis.

Several medications have the potential to cause salivary gland hypofunction and changes in saliva composition, but in contrast to chronic autoimmune diseases like Sjögren's syndrome and RT in the head and neck region, medication-induced salivary gland dysfunction is reversible. In patients with medication-induced salivary gland dysfunction, the number of mutans streptococci, lactobacilli and yeasts increases, and results indicate an increased risk of dental caries and oral candidiasis in these patients.

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Part III

Future Diagnostic Methods and Techniques

The Oral Microbiome in Health and Disease

10

Ingar Olsen

Abstract

This chapter deals with the human oral microbiome which contains bacteria, bacteriophages/viruses, archaea, fungi, and protozoa. Modern molecular techniques used to analyze this microbiome are dealt with such as HOMINGS, oligotyping, high-throughput sequencing, whole-genome shotgun sequencing, single-cell genome sequencing, metatranscriptomics, and community-wide transcriptome analysis. The oral microbiota in health is described as well as that in periodontal disease and dental caries. Furthermore, the architecture of biofilms in periodontitis and caries is visualized. Our knowledge on the oral microbiota challenges the current practice of chairside diagnostics.

10.1 The Human Oral Microbiome

The human oral microbiome is composed of a variety of different microorganisms such as bacteria, bacteriophages/viruses, yeasts, archaea, and protozoa. It has been suggested that these organisms cause diseases by a synergistic or cooperative way and that the interspecies interactions have a crucial role whether the oral microbiota causes disease or not (He et al. 2014). What is remarkable for this microbiota is also that its commensals contribute to disease, e.g., to caries and periodontitis through ecological changes. Another noteworthy feature is that it is personalized, meaning that each person harbors a unique microbiota. This implies that the human microbiome is more different between individuals than within an individual (Fig. 10.1). It has also been shown that characteristics of an individual's life history can be associated with the composition of the microbiome (Ding and Schloss 2014) and that the phylogenetic microbial structure varies with aging (Xu et al. 2014).

10.1.1 Bacteria

Bacteria have been considered the dominating part of the microbiome in man. However, while some six billion bacteria are present in the oral cavity, it

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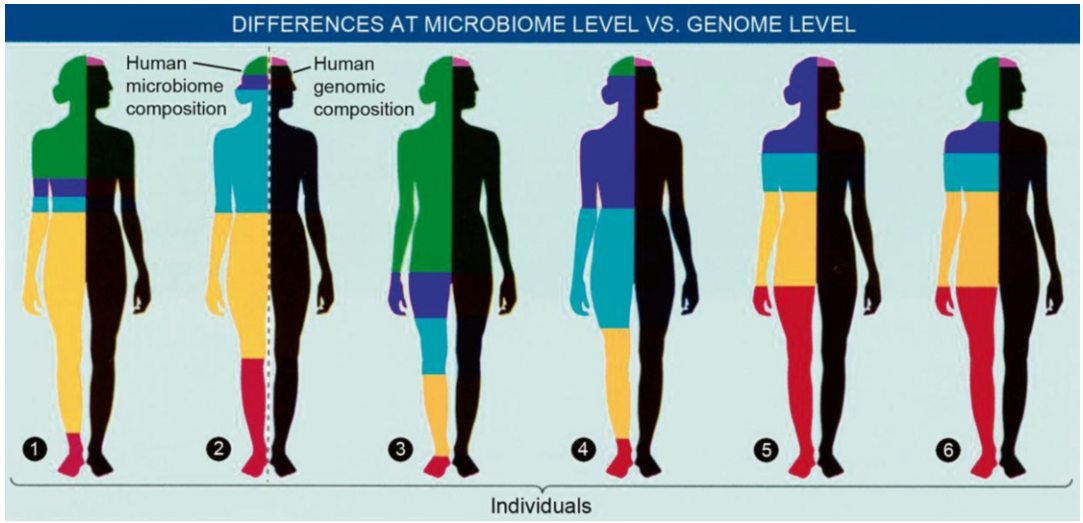

Fig. 10.1 Humans are far more different from each other in their microbial composition than in their genomic composition. The *colors* on the *left side* of each individual represent bacterial phyla, while the *colors* on the *right side* indicate host genomic similarity. For the most part we contain similar phyla living in and on our bodies, including the oral cavity, but their relative abundance can be drastically different. On the other hand, our genomic composition is nearly identical, with only a small fraction (ca 0.1 %) differing across individuals (Adapted from Califf et al. 2014)

contains potentially 35 times that many bacteriophages/viruses (Edlund et al. 2015). When Dewhirst et al. (2010) established the Human Oral Microbiome Database (HOMD) (http://www.homd.org/), it comprised over 600 prevalent bacterial taxa at the species level with distinct subsets predominating at different sites such as teeth, gingival sulcus, tongue, cheeks, hard/soft palate, and tonsils. The HOMD included 619 taxa from 13 phyla: Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Euryarchaeota, Firmicutes, Fusobacteria, Proteobacteria, Spirochaetes, SR1, Synergistetes, Tenericutes, and TM7. The analysis comprised 1179 taxa. Among these 24 % were named, 8 % were cultivated but unnamed, and 68 % were uncultivated phylotypes. Later the number of oral phyla has been extended to 15, but 96 % of the sequences are accounted for by only 6 phyla: Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria, and Spirochaetes (Wade 2013). Recently, Camanocha and Dewhirst (2014) developed primer pairs for making phylum-selective 16S rRNA clone libraries and identified species from the lesser known oral phyla or candidate divisions including Synergistetes, TM7, Chlorobi, Chloroflexi, GN02, SR1, and WPS-2.

10.1.2 Bacteriophages/Viruses

Oral viruses in saliva are dominated by bacteriophages (Pride et al. 2012). Also dental plaque is inhabited by a community of bacteriophages (Naidu et al. 2014). Bacteriophages constitute the major part of the oral virome with relatively few eukaryotic viruses identified such as herpesviruses, papillomaviruses, enteroviruses, and circoviruses (Grinde and Olsen 2010; Naidu et al. 2014). The mouth has been found to have more genetic elements than the stool, i.e., viruses, plasmids, and transposons, although it has fewer bacteria (Zhang et al. 2013). Bacteriophages may serve as reservoirs for genes functioning in the oral cavity. Phage members of the oral virome can carry genes involved in resistance to complement degradation of immunoglobulins, adhesion to cells lining the oropharynx, and antibiotic resistance (Pride et al. 2012; Muniesa et al. 2013; Abeles et al. 2014; Quirós et al. 2014).

Oral viruses have gene functions that may be involved in the pathogenic roles of their host bacteria (Pride et al. 2012). The same salivary viruses could be identified at all time points over 60 days despite being present in low numbers (Abeles

et al. 2014), reflecting that the oral viral ecosystem is stable. Most oral viruses are lysogenic and live in harmony with their hosts (Abeles and Pride 2014; Ly et al. 2014), and they may be important in shaping the microbial diversity of the oral cavity. Another peculiarity is that viral communities of the mouth are highly personalized (Willner et al. 2011; Pride et al. 2012), even more personalized than bacterial communities when analyzed with *16S rDNA* sequencing (Abeles et al. 2014). A noteworthy feature is also that oral viruses vary according to host sex, rather than among individuals (Abeles et al. 2014). The human oral viral community is probably a result of the unique viral exposures of each individual (Abeles et al. 2014), but considerably more of the oral virobiota of people living together is shared than could be expected by chance (Robles-Sikisaka et al. 2013). Eukaryotic viruses such as Torque Teno viruses (TTVs) and SEN viruses have been found in the bloodstream of healthy people (Pride et al. 2012; Abeles and Pride 2014). Blood of healthy persons have previously been considered sterile. Both these groups of viruses are present in the human oral cavity (Pride et al. 2012). Also herpesviruses, shed in the mouth from healthy individuals, can be found in human blood (De Vlaminck et al. 2013). Therefore not only bacteria but also viruses can translocate through mucosal surfaces to the bloodstream and possibly be involved in systemic diseases.

It is well known that the human oral cavity contains a large and diverse variety of bacteria. What viruses it contains has to a great extent been overlooked. This particularly relates to the periodontal microbiota, although herpesviruses including Epstein-Barr virus and cytomegalovirus can be present in high copy counts in aggressive periodontitis and may interact with periodontopathogenic bacteria to cause the disease (Sunde et al. 2008; Slots 2011; Contreras et al. 2014). Ly et al. (2014) examined samples from saliva of periodontally healthy and diseased patients and found that the communities of viruses inhabiting saliva and subgingival and supragingival biofilms were composed mainly of bacteriophages. The virome composition was greatly reflected by the site it was collected from. The largest difference in composition was between supra-/subgingival plaque and saliva. Differences in virus composition were significantly related to the health status of viruses in plaque, but not to those in saliva. Noteworthy, there was a significant increase in myoviruses (generally lytic) in subgingival biofilm suggesting that these viruses may have a great importance to local bacterial diversity and that the virus may serve as useful indicators of the oral health status. Since viruses have the potential to form microbial communities as well as to elicit host immune response, they probably play an important role in human health (Edlund et al. 2015). Also, the fact that they are personal, persistent, and gender specific suggests that they can be important in the interplay between host genetics and the environment.

10.1.3 Archaea

Archaea were originally considered a primitive form of life that thrives in extreme environments. However, high numbers of methane-producing archaea (methanogens) have now been detected in the oral cavity (Belay et al. 1988), the gastrointestinal tract (Karlin et al. 1982), and vagina (Belay et al. 1990) of human beings. The reported oral archaea contain the genera *Methanobrevibacter*, *Methanobacterium*, *Methanosarcina*, and *Methanosphaera* and the order Thermoplasmatales (He et al. 2014). The main species is *Methanobrevibacter oralis*. Archaea have been detected in saliva, periodontitis, peri-implantitis, pericoronitis, and infected root canals (Brusa et al. 1987; Belay et al. 1988; Kulik et al. 2001; Lepp et al. 2004; Vianna et al. 2006, 2009; Vickerman et al. 2007; Conway de Macario and Macario 2009; Jiang et al. 2009; Matarazzo et al. 2011, 2012; Faveri et al. 2011; Mansfield et al. 2012; Bringuier et al. 2013). These studies detected a higher frequency of archaea in oral infections than in health. Thus the relative abundance of archaea in subgingival plaque increased with the severity of periodontitis and decreased with the reduction of periodontitis after treatment. Archaea may therefore be associated

with periodontitis but the diversity of archaea is limited (Li et al. 2009). Almost all sequenced amplicons fell in the genus *Methanobrevibacter* of the Euryarchaeota phylum with *M. oralis*-like species as the most dominant. In root canal infections, presence of archaea was associated with clinical symptoms (Jiang et al. 2009). Although discussion of the clinical role of Euryarchaeota (including *Methanobrevibacter smithii*, *M. oralis*, and *Methanosphaera stadtmanae*) continues (Horz and Conrads 2010), and archaea are emerging organisms in complex human microbiomes (Dridi et al. 2011), methanogenic archaea do not seem to induce oral diseases directly. However, they may promote anaerobic infections through syntropic interactions with true pathogenic fermenting bacteria, e.g., through interspecies H_2 transfer, thereby favoring growth of certain bacteria (Matarazzo et al. 2012). Thus, a positive correlation has been found between methanogens and *Synergistes* species in oral infections (Vianna et al. 2006; Vartoukian et al. 2007).

10.1.4 Fungi

Dupuy et al. (2014) performed massive parallel, high-throughput sequencing of internal transcribed spacer 1 (ITS1) amplicons from saliva after robust extraction methods. Their findings confirmed nearly every community member from a similar study by Ghannoum et al. (2010) who had detected 74 cultivable and 11 non-cultivable fungal genera in the oral cavity by using multitag pyrosequencing of panfungal ITS primers. A consensus on genus-level members of oral fungi (core mycobiome) was thereby reached. This study was the first to demonstrate not-yet-cultivated fungi in the oral cavity. It was suggested that such organisms could be the reason for failure in the treatment of oral fungal infections. Consensus members of the saliva microbiome were *Candida*/*Pichia*, *Cladosporium*/*Davidiella*, *Alternaria*/*Lewia*, *Aspergillus*/*Emericella*/*Eurotium*, *Fusarium*/*Gibberella*, *Cryptococcus*/*Filobasidiella*, and *Aureobasidium*. Weaker candidates for consensus inclusion were *Saccharomyces*, *Epicoccum*, and *Phoma*. Interestingly, *Malassezia* species, that are important commensals of human skin, were for the first time included in the oral core mycobiome. The oral fungal community showed a consistent intraindividual stability over time, but there was high interindividual variability (Monteira-da-Silva et al. 2014).

Interactions between fungi and bacteria, e.g., between *Candida* and streptococci, may influence oral health (Diaz et al. 2014). A symbiotic relationship between *S. mutans* and *C. albicans* has been found to synergize virulence of plaque biofilms in vivo (Falsetta et al. 2014). Thus *S. gordonii* glucosyltransferase promotes biofilm interactions with *C. albicans* (Ricker et al. 2014). Fungi probably have a role in maintaining a balance between microorganisms and the host (Krom et al. 2014).

10.1.5 Protozoa

Protozoa are parts of the normal microbiome. The best known are *Entamoeba gingivalis* and *Trichomonas tenax* (Vozza et al. 2005). They are present in subjects who neglect their oral hygiene and predominantly in subgingival plaque from patients with periodontal disease (Lange et al. 1983). Both have been linked to gingivitis and they were once considered pathogens. *T. tenax* has been correlated with xerostomia, burning mouth, and periodontal pockets (Kurnatowska 1993; Kurnatowska and Kurnatowski 1998). Later, it has become clear that these organisms increase when the oral hygiene deteriorates. Their increase may be due to nutrients accessible from debris and bacteria (Wade 2013). It is interesting though that metronidazole, frequently used as an effective supplement in the treatment of periodontitis, is active against both *Entamoeba* and *Trichomonas*.

10.2 Techniques to Analyze the Oral Microbiota

It should be realized that every technique that has been used to detect oral microorganisms has its strengths and limitations. Not all of these techniques will be dealt with here. Microscopy and culture were long standard methods for assessment of the oral microbiota. Later, culture helped

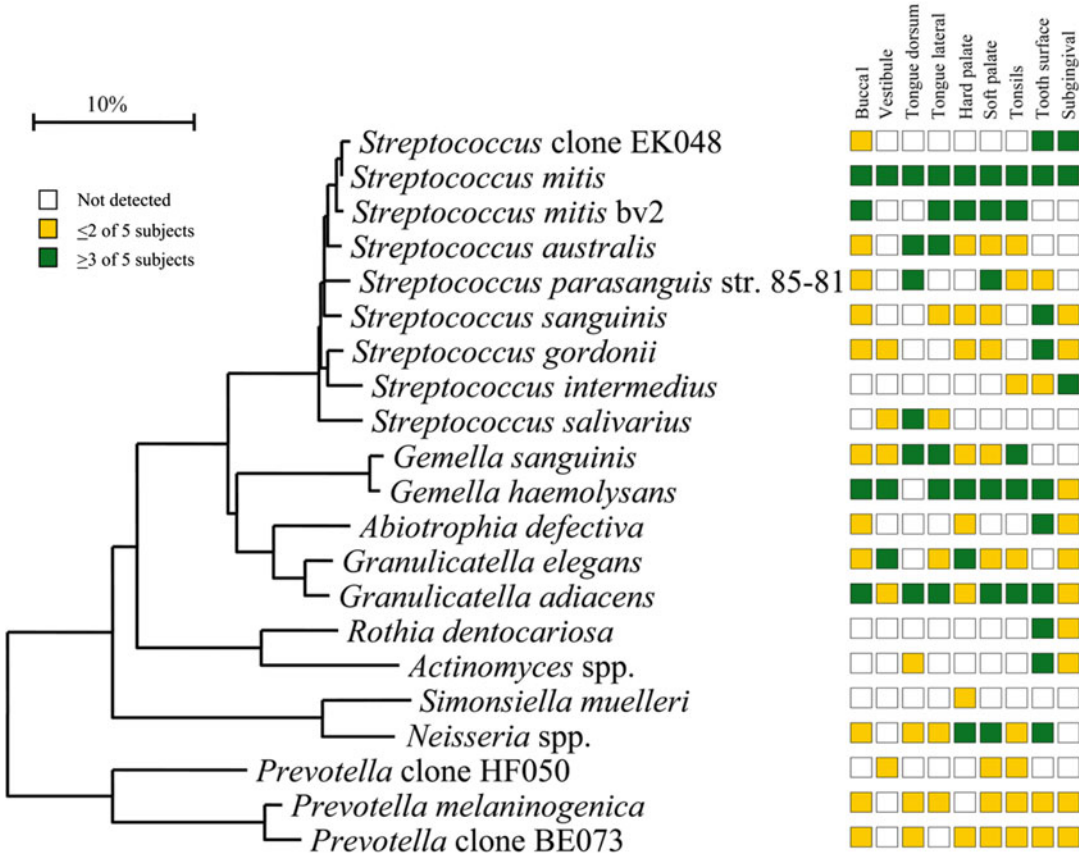

Fig. 10.2 Site specificity of predominant bacterial species in the mouth. Bacterial species or phylotypes were selected on the basis of their detection in multiple subjects for a given site. Distributions of bacterial species in oral sites among subjects are indicated by the columns of boxes to the right of the tree as follows: not detected in any subject (*clear box*), < 15 % of the total number of clones assayed (*yellow box*), and ≥ 15 % of the total number of clones assayed (*green box*). The 15 % cutoff for low and high abundance was chosen arbitrarily. Marker bar represents a 10 % difference in nucleotide sequences (From Aas et al. 2005)

us become more familiar with this microbiota when methods for recovery of anaerobic bacteria were developed. However, it soon became clear that only half of the oral microbiota could be cultured. Therefore culture-independent methods were exploited, particularly DNA-DNA hybridization and PCR-based assays. DNA-DNA hybridization (checkerboard) relied though on bacteria that could be cultivated for the making of whole genomic probes (Socransky et al. 1994), but reverse-capture checkerboard hybridization did not (Paster et al. 1998). Checkerboard DNA-DNA hybridization was helpful delineating bacteria clinically related to periodontitis such as the red and the orange complex (Socransky et al. 1998). Since there was reason to believe that also not-yet-cultivated bacteria could be involved in disease methods, targeting the small subunit (16S) ribosomal RNA molecule was used. These efforts have provided a vast amount of knowledge and description of the oral microbiota. They have also shown that the oral microbiota is not uniform but varies from site to site (Fig. 10.2). The information has been collected in the first curated collection of a human-associated microbiome, HOMD, which provides a description of the organisms and their genomics together with a 16S rRNA identification tool (Dewhirst et al. 2010), and later in the CORE database that is a phylogenetically curated *16S rDNA* database of

the core oral microbiome (Griffen et al. 2011). Although *16S rRNA* gene amplification and Sanger sequencing significantly increased our knowledge of the major components of the oral microbiota, they did not provide information of the entire microbiota. Organisms that are present in low amounts were first revealed by pyrosequencing (next-generation sequencing methods).

10.2.1 HOMINGS

HOMINGS (http://homings.forsyth.org) apply the speed and efficiency of the next-generation sequencing using the Illumina platform. Almost 600 oral bacterial taxa can be identified with this technique which provides genus-level identification of the remaining sequences for 129 genera. It is thus more comprehensive than its predecessor HOMIM which gave simultaneous microarray detection of about 270 of the most prevalent, cultivated, and not-yet-cultivated oral bacterial species.

10.2.2 Oligotyping Analysis of the Human Oral Microbiome

A limited taxonomic resolution has often prevented understanding the census of bacterial populations in healthy individuals. By using *16S rRNA* gene sequence data from nine sites in the oral cavity, Eren et al. (2014) identified 493 oligotypes from their V1-V3 data and 360 oligotypes from the V3-V5 data. The oligotypes were associated with species-level taxon names by comparing with HOMD. The authors discovered closely related oligotypes differing sometimes by only a single nucleotide that showed widely different distributions among oral sites and samples. Different habitat distributions of closely related oligotypes indicated a level of ecological and functional biodiversity not recognized previously. This technique combined with Shannon entropy has the capacity to analyze entire microbiomes and discriminate between closely related but distinct taxa in different habitats.

10.2.3 High-Throughput Sequencing (Pyrosequencing)

16S rRNA sequencing using next-generation sequencing has provided a wealth of new knowledge on the genetic composition of the oral microbiome in health and disease. The most useful of these approaches have relied on the 454 (Roche) pyrosequencing platform. In Table 10.1, the advantages and limitations of different high-throughput sequencing platforms are summarized.

10.2.4 Whole-Genome Shotgun Sequencing

Whole-genome shotgun sequencing (WGS) can provide highly accurate sequences in an economic way and has a fast turnaround (Hasan et al. 2014). WGS metagenomic sequencing has proved to be a powerful tool for studying the human microbiome. At present, WGS metagenomic data contain millions to billions of short reads and offer an unprecedented opportunity to identify species at or near strain level and their abundance.

10.2.5 Single-Cell Genome Sequencing

Remarkable in the identification of bacteria is single-cell genome sequencing which enables not only identification of microbes but links their functions to species, which is not feasible with metagenomic techniques. It also analyzes low-abundance species that can be lost in community-based analyses and can be useful in complementing metagenomic analyses (Yilmaz and Singh 2012). An ultimate goal of single-cell sequencing is recovery of genome sequences from each cell within an environment (Clingenpeel et al. 2015).

10.2.6 Metatranscriptomics of the Oral Microbiome during Health and Disease

Although new techniques have revealed what organisms are present in the oral microbiome,

Table 10.1 Comparison of next-generation sequencing platforms

Machine (manufacturer)	Chemistry	Modal read length[a] (bases)	Run time	Gb per run	Current, approximate cost (US$)[b]	Advantages	Disadvantages
High-end instruments							
454 GS FLX+ (Roche)	Pyrosequencing	700–800	23 h	0.7	500,000	Long read lengths	Appreciable hands-on time High reagent costs High error rate in homopolymers
HiSeq 2000/2500 (Illumina)	Reversible terminator	2×100	11 days (regular mode) or 2 days (rapid run mode)[c]	600 (regular mode) or 120 (rapid run mode)[c]	750,000	Cost-effectiveness Steadily improving read lengths Massive throughput Minimal hands-on time	Long run time Short read lengths HiSeq 2500 instrument upgrade not available at time of writing (available end 2012)
5500xl SOLiD (Life Technologies)	Ligation	75+35	8 days	150	350,000	Low error rate Massive throughput	Very short read lengths Long run times
PacBio RS (Pacific Biosciences)	Real-time sequencing	3000 (maximum 15,000)	20 min	3 per day	750,000	Simple sample preparation Low reagent costs Very long read lengths	High error rate Expensive system Difficult installation
Bench-top instruments							
454 GS Junior (Roche)	Pyrosequencing	500	8 h	0.035	100,000	Long read lengths	Appreciable hands-on time High reagent costs High error rate in homopolymers
Ion Personal Genome Machine (Life Technologies)	Proton detection	100 or 200	3 h	0.01–0.1 (314 chip), 0.1–0.5 (316 chip), or up to 1 (318 chip)	80,000 (including OneTouch and server)	Short run times Appropriate throughput for microbial applications	Appreciable hands-on time High error rate in homopolymers

(continued)

Table 10.1 (continued)

Machine (manufacturer)	Chemistry	Modal read length[a] (bases)	Run time	Gb per run	Current, approximate cost (US$)[b]	Advantages	Disadvantages
Ion Proton (Life Technologies)	Proton detection	Up to 200	2 h	Up to 10 (Proton I chip) or up to 100 (Proton II chip)	145,000 + 75,000 for compulsory server	Short run times Flexible chip reagents	Instrument not available at time of writing
MiSeq (Illumina)	Reversible terminator	2 × 150	27 h	1.5	125,000	Cost-effectiveness Short run times Appropriate throughput for microbial applications Minimal hands-on time	Read lengths too short for efficient assembly

Adapted from Loman et al. (2012)
[a]Average read length for a fragment-based run
[b]Approximate cost per machine plus additional instrumentation and service contract
[c]Available only on the HiSeq 2500

they do not tell anything about the viability of the organisms or their functions. Therefore efforts have been made recently to use microbiomics, metagenomics, and transcriptomics to better understand the role of the oral microbiome in health and disease. This may also help us to more efficiently prevent these diseases and provide a personalized treatment.

Our indigenous microbiota is closely linked to health. However, when disrupted the same microbiota can induce disease. Such diseases are characterized by changes in the relative amounts of different species. While such changes in the microbiota occur, it is also clear that the members of the microbial communities can differ markedly between individuals (Ge et al. 2013). This applies to the microbiota of both healthy and diseased individuals. In a study based on nine patient-matched healthy and diseased samples, 160,000 genes were compared in healthy and diseased periodontal communities (Jorth et al. 2014). Massive parallel RNA sequencing was used to demonstrate changes in the composition and gene expression of the microbiota in health and periodontitis. It was shown that both communities exhibited defined differences in metabolism that were conserved between patients. In contrast, the metabolic gene expression of individual species within the community varied greatly between patients. Disease-associated communities also showed conserved changes in metabolic and virulence gene expression. Thus, by using transcriptional profiling the authors could determine changes in the composition and gene expression of the human oral microbiota in health and periodontitis.

By using metatranscriptome analysis of periodontal biofilm in vitro, it was demonstrated that addition of periodontal pathogens to a healthy biofilm multispecies model had a drastic effect in changing the gene expression profiles of the organisms of the healthy community (Frias-Lopez and Duran-Pinedo 2012). Chaperones were highly upregulated, possibly due to stress, and there was a significant upregulation of ABC transporter systems and putative transposases. With pathogens present, proteins related to growth and division, as well as a large portion of transcription factors, were upregulated.

10.2.7 Community-Wide Transcriptome Analysis of the Oral Microbiome in Subjects With and Without Periodontitis

Our knowledge on the in situ activities of the organisms and their interaction with each other and with the environment is limited. Such knowledge may be obtained by characterizing gene expression profiles of the microbiome. In situ genome-wide transcriptome variation was studied in the subgingival microbiome of six periodontally healthy individuals and seven individuals with periodontitis (Duran-Pinedo et al. 2014). The overall metabolic activities defining disease were related to iron acquisition, lipopolysaccharide synthesis, and flagella synthesis. It was both noteworthy and unexpected that the majority of virulence factors upregulated in periodontitis came from organisms not considered as major pathogens. Also remarkable was that one of the organisms with characterized gene expression profile was from the uncultured candidate division TM7 exhibiting upregulation of putative virulence factors in disease. This demonstrated the importance of in situ metatranscriptomic studies for studying the possible etiological role of uncultured organisms. Unexpectedly, no viral sequence was detected in either the metagenome or the metatranscriptome.

10.3 Oral Microbiota in Health

The oral microbiota in health is highly diversified. It consists of approximately 600 predominant species (Dewhirst et al. 2010) that contribute to the health and physiology of the oral cavity. Two main types of tissues are colonized: soft and hard tissues. It is also clear that the oral cavity contains different niches for bacterial growth with different bacterial profiles that are site and subject specific (Fig. 10.2). Even close sites such as the dorsal and lateral sides of the tongue dorsum (Aas et al. 2005) and the vestibular and lingual surfaces of incisors and canines (Simon-Soro et al. 2013) have different microbiotas. The oral microbiota has, due

to its continuum with the external environment, developed features to counteract challenges from foreign bacteria. There is probably a core microbiome for health which is common to all individuals (Zarco et al. 2012). In addition, there is a variable microbiome unique to individuals depending on lifestyle and physiological differences. Supporting the existence of a core microbiome was that identical bacterial sequences were detected in the oral cavities of unrelated healthy persons (Zaura et al. 2009). Transcription profiling defined a functional core microbiota of nearly 60 species in dental plaque (Peterson et al. 2014), and Wang et al. (2013) described a core disease-associated community in periodontitis by metagenomic sequencing. A study based on a large set of near full-length sequences in 10 healthy individuals identified 10 variables shared by 11 bacterial species (Bik et al. 2010). However, there were also significant inter-individual differences. This supported the presence of both a core and a variable microbiome in the oral cavity. Based on several literature reports (Zarco et al. 2012) the major genera with the largest representation in the oral cavity were found to include *Streptococcus*, *Veillonella*, *Granulicatella*, *Gemella*, *Actinomyces*, *Corynebacterium*, *Rothia*, *Fusobacterium*, *Porphyromonas*, *Prevotella*, *Capnocytophaga*, *Neisseria*, *Haemophilus*, *Treponema*, *Lactobacterium*, *Eikenella*, *Leptotrichia*, *Peptostreptococcus*, *Staphylococcus*, *Eubacteria*, and *Propionibacterium*.

10.3.1 Microbiota in Periodontal Disease

Over the years, there have been several milestones and hypotheses on the microbial etiology of periodontitis (Hajishengallis and Lamont 2012). Etiologies related to specific organisms (amoeba, spirochetes, fusiforms, or streptococci), nonspecific plaque hypothesis/mixed anaerobic infections, microbial shift in periodontitis, specific plaque hypothesis, red complex bacteria (*Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*), ecological catastrophe hypothesis, disruption of periodontal tissue homeostasis, keystone pathogens, and polymicrobial synergy and dysbiosis (PSD) can be mentioned. This variability may partly be considered results of increased knowledge related to instrumental analytical improvements. However, rather than mentioning the microorganisms involved under each etiological heading, space will be devoted here to the most recent concept, PSD.

In the PSD model, it is recognized that the gingival crevice is colonized by a diverse microbiota where compatible microorganisms assemble into heterotypic communities. These are in equilibrium with the host. The organisms are controlled by the host, despite their production of toxic products such as proteases, overgrowth, and pathogenicity. Noteworthy, the microbial components of these communities vary over time from person to person and from site to site. The virulence of the entire community is increased by keystone pathogens such as *P. gingivalis* which can have interactive communication with accessory pathogens like the *mitis* group of streptococci, thereby orchestrating inflammatory disease by remodeling a normally benign microbiota into a dysbiotic one (Hajishengallis and Lamont 2012; Hajishengallis et al. 2012). The host immune response is not impaired and the abundance of the dysbiotic community increases, destroying tissue homeostasis and causing destruction of periodontal tissues. PSD is probably not the last model of periodontitis that will be launched, but it is attractive from the point that it reconciles the joint effects of a synergetic and a dysbiotic microbial community, rather than select organisms.

In terms of the microorganisms related to periodontitis, it should be mentioned that it is now moderate evidence in the literature to support the association of 17 species or phylotypes from the phyla Bacteroidetes, Candidatus Saccharibacteria, Firmicutes, Proteobacteria, Spirochaetes, and Synergistetes with periodontitis. Also the archaea domain seems to have an association with this disease (Pérez-Chaparro et al. 2014). As already mentioned, every human body carries a personalized microbiome that is important for maintaining health but also for eliciting disease (Zarco et al. 2012; Califf et al. 2014). According to Schwarzberg et al. (2014) who used

next-generation sequencing, there is not a single microbial composition that represents a healthy periodontal state and that recovery from periodontal disease appears to shift from a personalized disease state to a personalized healthy state. Although there may be a consensus that particular communities will shift according to disease, there may not be a healthy part of these bacteria that is consistent across individuals. In contrast to this Griffen et al. (2012), using 16S multiple region pyrosequencing, found differences between health- and periodontitis-associated bacterial communities at all phylogenetic levels and distinct community profiles. Spirochaetes, Synergistetes, and Bacteroidetes were prominent phyla in disease, while Proteobacteria was detected at higher levels in healthy controls. Their data confirmed the association of species such as *P. gingivalis*, *T. denticola*, and *T. forsythia* with disease, but *Filifactor alocis* appeared to be at least as prevalent and disease associated. Abusleme et al. (2014), using 454 pyrosequencing of *16S rRNA* gene libraries, found that periodontitis communities were high in Spirochaetes, Synergistetes, Firmicutes, and Chloroflexi among other taxa, while the proportion of Actinobacteria, especially *Actinomyces*, was more abundant in health.

A number of bacterial taxa and genes have been found to differ between health and disease. Until now data sets across studies have not been compared directly, and we do not know if the microbial variations observed across studies are consistent. Kirst et al. (2015) used 16S rRNA sequencing to survey the subgingival microbiota in 25 subjects with chronic periodontitis and 25 controls and compared their data with those of the Human Microbiome Project (HMP) (Turnbaugh et al. 2007; Griffen et al. 2012; Abusleme et al. 2013). They found a significantly altered microbiota with decreased heterogeneity in periodontal disease. Comparison with the other data sets showed that the subgingival microbiota clustered by study. However, differences between periodontal health and disease were greater than the technical variations between the studies. Two microbial clusters were detected. One was driven by *Fusobacterium* and *Porphyromonas* and was associated with periodontitis; the other consisted of *Rothia* and *Streptococcus* and was related to health.

In a study by Ly et al. (2014), the oral bacteriophage membership was significantly changed in persons with periodontitis compared to healthy subjects, mainly as a result of abundance of myoviruses in subgingival plaque. Myoviruses are mainly lytic. Their predominance in subjects with periodontitis suggested an active role for viruses in driving bacterial diversity in the periodontal pocket. They were more abundant than siphoviruses which generally have a lysogenic lifestyle. In supragingival plaque, however, there was no difference between myoviruses and siphoviruses. The altered ecology suggested for bacterial involvement in periodontitis could therefore also involve bacteriophages.

10.3.2 Biofilm Architecture in Periodontitis

Sampling of dental plaque will destroy its architecture making it difficult to conclude firmly on the relative pathogenic role of taxa. When different materials were kept for several days in periodontal pockets of patients with periodontitis and examined with electron microscopy and fluorescence in situ hybridization (FISH), those parts of carriers extending into the deepest zone of the pocket were mainly colonized by spirochetes and Gram-negative bacteria (Wecke et al. 2000). Those kept in shallower regions were colonized by streptococci. The methods allowed detailed analysis of the architecture of biofilms and identification of putative periodontal pathogens with single-cell resolution. Previous investigations had revealed presence of novel yet uncultivated organisms at a high frequency in periodontal pockets (Moter et al. 1998). All patients with rapidly progressive periodontitis ($n = 53$) harbored oral treponemes that were either new species such as *T. maltophilum* or uncultivable phylotypes. When enamel slices were used to examine the microbiota development of dental plaque, channels or pores filled with extracellular polymers were seen throughout the biofilm (Wood et al. 2000).

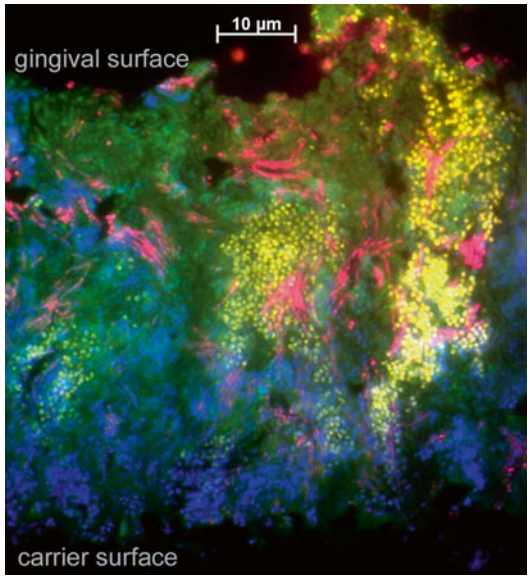

Fig. 10.3 Fluorescence in situ hybridization (FISH) of a subgingival biofilm showing the close spatial relationship between facultatively anaerobic *Streptococcus* spp. (*orange*) and obligately anaerobic *Fusobacterium* spp. (*magenta*). Subgingival biofilms of periodontitis patients were obtained using a carrier system. Bacteria were visualized in 3 µm cross sections of the biofilms using the following probes simultaneously: probe EUB338, which detects most bacteria (*green*); probe Strep1/2, which shows streptococci; probe FUS664, which detects most *Fusobacterium* spp.; and nonspecific nucleic acid stain DAPI (*blue*). Details of oligonucleotide probes are available at probeBase (http://www.microbial-ecology.net/probebase/) (From Marsh et al. (2011) with permission)

Staining and confocal microscopy showed that the most viable and active areas of the biofilm were in the central parts and parts lining the channels. Plaque biofilms in the gingival crevice had a thin densely adherent layer on the surface of the root, while the bulk of the biofilm had a looser structure particularly where there was contact with the epithelial lining of the gingival crevice or periodontal pocket (Fig. 10.3). In outer layers structures such as corncob, test-tube brush, or rosette formations were detected together with not-yet-cultivated organisms such as spirochetes and members of the TM7 phylum (Fig. 10.4). In the plaque itself interacting bacteria exhibited a spatial organization, e.g., between streptococci and *Fusobacterium nucleatum*.

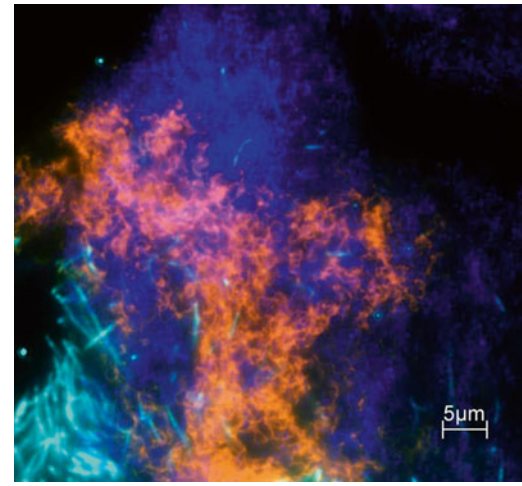

Fig. 10.4 High numbers of group I treponemes (*orange*) in a subgingival biofilm, most of which are yet uncultured. The carrier section was hybridized with probe TRE I together with FUNU for detection of *Fusobacterium nucleatum/canifelinum* (*light blue*), which forms a cluster in the lower left corner, and DAPI (*dark blue*) (From Marsh et al. (2011) with permission)

10.3.3 Bacteria Associated with Caries

Recent theories divide the dental caries process into three reversible stages: the dynamic stability stage, the acidogenic stage, and the aciduric stage (Takahashi and Nyvad 2008, 2011; Nyvad et al. 2013). The microbiota on clinically sound enamel consists mainly of non-*mutans* streptococci and *Actinomyces*. Here acidification is mild and infrequent which is reflected in a balanced demineralization/remineralization or a shift in the mineral balance toward a net mineral gain (dynamic stability stage). Acidification becomes moderate and frequent when sugar is added. This may increase the acidogenicity and aciduranceof non-*mutans* bacteria. There can also be a selective increase in more aciduric strains such as low pH non-*mutans* streptococci. In the end, this will shift the demineralization/remineralization balance, so that a net mineral loss occurs, leading to initiation/progression of dental caries (acidogenic stage). If the acidogenic conditions become severe and prolonged, aciduric bacteria will predominate by acid-induced selection

(aciduric stage). At this stage, *mutans* streptococci, lactobacilli, aciduric strains of non-*mutans* streptococci, *Actinomyces*, bifidobacteria, and yeasts may become dominant.

Different components of the microbiota may play different roles in initial enamel lesions compared to caries extension into dentin. The hydroxyapatite-rich enamel likely requires a more acidic microbiota for demineralization than dentin. The highly acidogenic species include *S. mutans*, acidogenic non-*mutans* streptococci, *Actinomyces* species, and *Bifidobacterium/Scardovia* species (Chalmers et al. 2015), whereas caries progression into dentin may involve proteolysis by *Prevotella* species of proteins denatured by acidic species (Hashimoto et al. 2011). It seems likely that the proteolytic component also will lead to pulp tissue necrosis considering the frequent detection of Gram-negative taxa in root canal infections.

As for the microorganisms involved in caries, direct pyrosequencing of samples from dental cavities showed that cavities are not dominated by *S. mutans* but contain a complex community of bacterial species (Belda-Ferre et al. 2012). This supported previous 16S rRNA sequencing studies (Corby et al. 2005; Aas et al. 2008) and the idea that dental caries is a polymicrobial disease. Pyrosequencing also supported that oral bacteria are specific at different stages of caries progression (Jiang et al. 2014). In children with severe dental caries, the genera *Streptococcus*, *Granulicatella*, and *Actinomyces* had increased significantly (Jiang et al. 2013).

By performing comprehensive 16S DNA profiling of the dental plaque microbiome of both caries-free and caries-active microbiomes, the signatures associated with dental health outnumbered those associated with dental caries by nearly twofold (Peterson et al. 2013). It was suggested that a shift in the abundance of groups of species, rather than the appearance of new cariogenic species or the pathogenicity of a single species, best describes the distinction between caries-free and caries-active microbiota.

Detection of major bacteria present in dental caries needs to be followed by information on the metabolic activity of the biofilm. Therefore, approaches such as metagenomic, metatranscriptomic, metaproteomic, and metabolomic analysis should be used to provide better information on the dynamic caries process. The precise determination of function requires the analysis of individual cells and cultures. In this context, it is important that previously uncultured microorganisms are being brought to culture. Emphasis should also be made to obtain site-specific sampling of microbial communities for studying the molecular ecology in situ of caries (Dige et al. 2014).

The next-generation sequencing technique was combined with a metagenomic technique and showed that individuals who had never suffered from caries had an overrepresentation of functional genetic categories such as genes for antimicrobial peptides and quorum sensing. They did not carry *mutans* streptococci (Belda-Ferre et al. 2012). Interestingly, several isolates belonging to healthy conditions inhibited the growth of cariogenic bacteria when they were co-cultured. Thus, the metagenomic approach enabled quantitation of the most abundant bacteria and confirmed presence of bacteria with a protective effect against cariogenic species.

10.3.4 Architecture of Biofilms in Caries

In occlusal caries, FISH showed a distinct difference in the bacterial composition between different ecological niches in the caries process (Dige et al. 2014). Biofilms located at the entrance of fissures had an inner compact layer of microorganisms structured in palisades often with a columnar pattern (Fig. 10.5). They were often identified as *Actinomyces* and were covered by a loosely structured bacterial layer consisting of various genera that were similar to supragingival biofilm. Within the proper fissure the biofilm appeared less metabolically active as estimated from low fluorescence signal intensity and presence of material of nonbacterial origin. Invasion of bacteria, often *Lactobacillus* and *Bifidobacterium* spp., into dentinal tubules was seen only at advanced stages of caries with cavity formation.

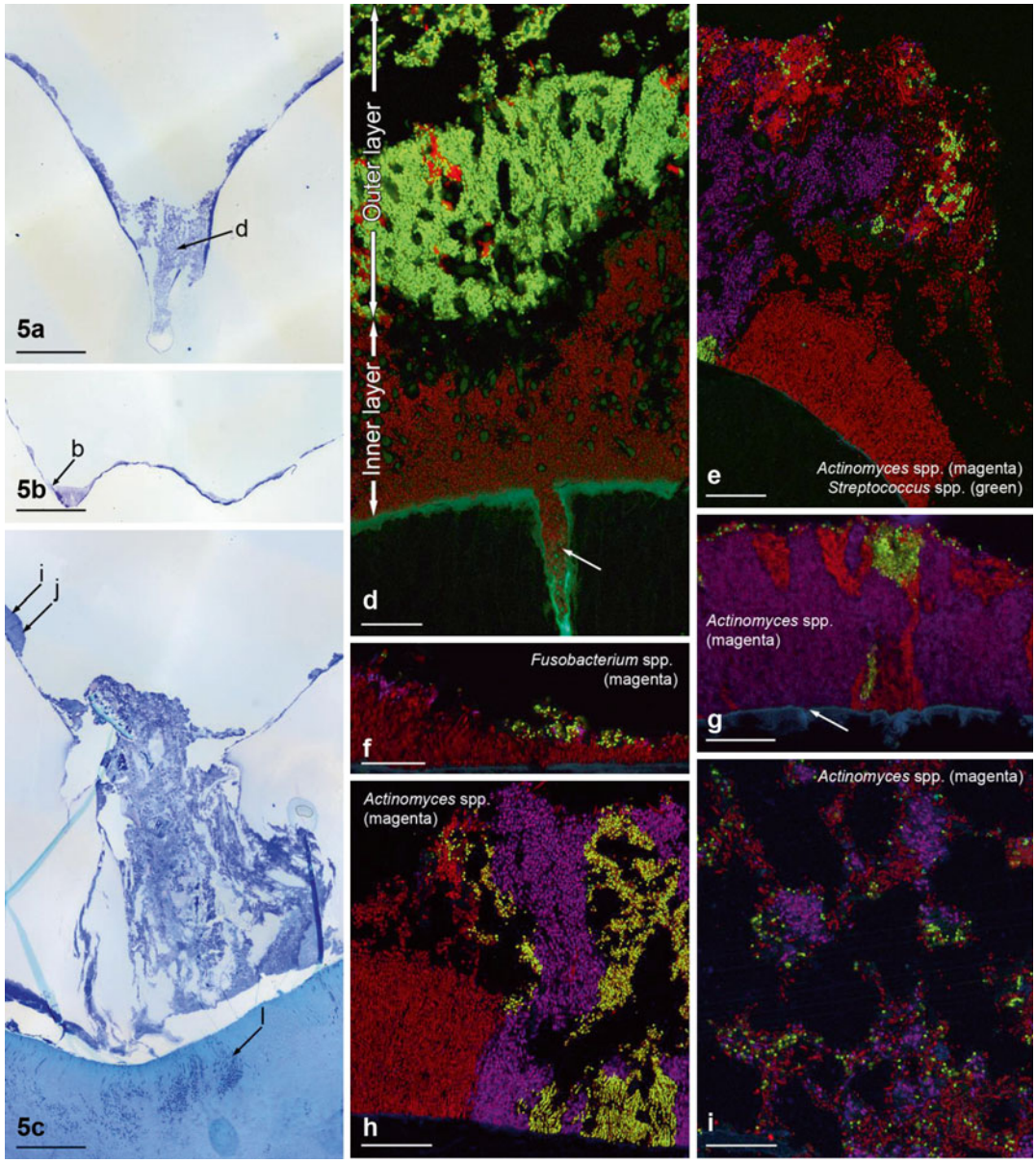

Fig. 10.5 (**a**–**c**) Images of in vivo biofilms on dental occlusal surfaces. (**a**–**c**) Toluidine blue-stained sections showing an overview of occlusal surfaces with shallow fissure-like morphology (**a**), groove-like morphology, (**b**) and cavitated caries lesion (**c**). *Arrows* refer to the areas illustrated in *b*, *d*, *i*, *j*, and *l*, respectively. (**d**–**i**) Confocal laser scanning microscopy images of microbial colonization patterns from above the entrance of shallow fissures and groove-like occlusal surfaces. In all confocal laser scanning microscopy images, *red* represents all bacteria that are neither *Streptococcus* spp. (*yellow*/*green* in **d**–**i**) nor *Actinomyces* spp. (*purple*/*magenta* in **e**, **g**–**i**) nor *Fusobacterium* spp. (*purple*/*magenta* in **f**). Note that the biofilm could be divided into an inner compact layer of palisade-like bacteria (**d**–**h**) often with a columnar pattern (**g**, **h**) on top of which a looser structured layer (**d**, **e**, **f**, **h**, **i**) with non-stained voids (**d**, **i**) was seen. The outermost part of the decalcified enamel showed a thin autofluorescent layer without bacteria (*blue* or *green* in **d**, **g**), and invaginations of developmental origin were often filled with bacteria (**d**, **g**, *arrows*). All images are oriented with the biofilm surface upward. Scale bars: 500 µm **a**–**c**) and 25 µm (**d**–**i**) (Adopted from Dige et al. (2014) with permission)

10.3.5 Future Chairside Diagnostics of Dental Plaque

Molecular studies have informed us about the great complexity of the oral microbiota both in health and disease, and we have been able to study microbial communities on a large scale due to advancements in sequencing and bioinformatics. They have also pointed out that specific organisms are not responsible for disease but rather rely on the supplementary action of other organisms. Recent studies have further taught us that a species can comprise strains of different virulence. This throws doubt on the species as a reasonable diagnostic unity (Wade 2013). The development of molecular diagnostics has been so fast that it seems reasonable now to turn to functions of the microbiota, rather than to what organisms are present. This makes a great challenge to chairside diagnostics of dental plaque which should try to implement the new knowledge into their procedures, rather than focus on a handful of select organisms.

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Salivary Microbiota in Oral Health and Disease

11

Daniel Belstrøm

Abstract

The salivary microbiota is a highly complex microbial community, presumably comprised by bacteria shed from various oral surfaces, and since saliva can be easily and noninvasively collected, compositional changes of the salivary microbiota may potentially serve as a biomarker used for screening of oral and systemic diseases. This chapter describes the composition of the salivary microbiota in oral health and disease, with special emphasis on recent studies that employed modern molecular methods for analysis of the salivary microbiota.

11.1 Salivary Microbiota in Oral Health

The salivary microbiota contains a unique individualized bacterial community shaped by contentious interaction with the surroundings, as bacteria are shed from different oral locations and captured in saliva. Interestingly, 1 ml of saliva contains >100 million bacteria, which means that a person with a normal saliva flow of 750 ml will excrete >5 g of solid bacteria through saliva in 24 h (Curtis et al. 2011). However, although the salivary microbiota is highly diverse, bacterial DNA has been reported to constitute only 0.73 % of total DNA present in saliva (Lazarevic et al. 2012).

The composition of the salivary microbiota in oral health has been addressed in detail. A comprehensive study from 2012 performed as part of the Human Microbiome Project analyzed and compared bacterial community profiles in samples collected from 10 different sites of the digestive tract in each of more than 200 healthy individuals (Segata et al. 2012). The study reported that the salivary microbiota in oral health mostly resembled that of the throat, the tonsils, and dorsum of the tongue. Firmicutes, Bacteroidetes, Proteobacteria, and Fusobacteria were described as the predominant phyla constituting 40 %, 25 %, 20 %, and 10 % of the microbiota, respectively. The predominant genera were *Streptococcus* and *Veillonella* accounting for 20 and 15 % of the microbiota in saliva. Interestingly, major differences between supra- and subgingival

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microbiota and salivary microbiota were evident, indicating that bacteria shed from these surfaces only accounts for a minor fraction of the salivary microbiota in oral health. Recently, next-generation sequencing was used to investigate saliva from two adults, and five datasets from the Human Microbiome Project, and identified more than 175 bacterial species present in saliva in oral health. Furthermore, it was estimated that the predominant microbiota of saliva comprised as much as 900 different bacterial species (Hasan et al. 2014), indicating that the salivary microbiota is probably far more complex than hitherto anticipated. In that respect, an interesting finding was reported in 2014, as an investigation of saliva samples from 97 healthy children aged 6–12 years revealed that 43 % of the samples were culture-positive for *Streptococcus aureus*, and in 6 % of the samples, *S. aureus* were identified at a level of $>10^3$ CFU/mL, proposing that saliva may harbor low proportions of pathogens as commensal members of the salivary microbiota in oral health (Petti et al. 2014).

It has been suggested that composition of the salivary microbiota is influenced by geographic location and individual lifestyle. To elucidate this perspective, an extensive study from 2010 analyzed saliva samples collected from 120 individuals living in each of 12 different locations worldwide, i.e., 10 individuals from each location, by means of 16S rRNA sequencing. A total of 101 genera were identified, and out of these 39 were not previously identified in the oral cavity. Furthermore, the study reported a major diversity of the salivary microbiota, but it was concluded that this diversity was only minimally influenced by geographic location, since the diversity among individuals from the same location was almost the same as between individuals living at different locations (Nasidze et al. 2009). Another report by the same group addressed composition of salivary microbiota in samples collected from two geographically different industrialized African populations and compared these with saliva samples from *Batwa Pygmies*, an isolated hunter society from Uganda. This study revealed that the salivary microbiota from the *Batwa Pygmies* expressed significantly more diversity than samples from the corresponding industrialized individuals and also reported finding of 40 bacterial genera in samples from *Batwa Pygmies* that had not previously been identified in the oral cavity. Therefore, the authors suggested that the differences observed could be a result of a different diet and lifestyle between the populations investigated (Nasidze et al. 2011). Analogous, in a recent publication from 2014, data based on Human Oral Microbe Identification Microarray (HOMIM) analysis of saliva samples from 293 orally healthy adult Danes suggested that saliva microbiota in oral health is influenced by individual smoking habit as well as socioeconomic status. However, in this particular study, no association was identified between different dietary habits and composition of the salivary microbiota (Belstrøm et al. 2014c). Thus, future studies are warranted to further elucidate the impact of diet and lifestyle on the composition of the salivary microbiota. The oral microbiota, including that of saliva, is developing from the moment of birth, and it has been hypothesized that during fetal life the fetus may in fact be exposed to the oral microbiota, thereby facilitating an ability of the newborn to better distinguish microbial friend from foe after birth (Zaura et al. 2014). Interestingly, the salivary microbiota has been demonstrated to be relatively simple in a newborn child, and salivary microbiota has been reported insignificant from stool microbiota of the same newborn until 15 days after birth (Costello et al. 2013). Furthermore, a delayed compositional differentiation of the salivary microbiota in low-birth-weight children has been demonstrated (Costello et al. 2013). In a recent study, saliva samples from newborn babies and saliva samples from their mothers/primary caregivers were collected and compared, and based on the data presented, high diversity of salivary microbiota in both study groups were evident. However, higher diversity was reported in samples from adults, as 27 genera were observed with greater prevalence in saliva from adults, whereas *Streptococcus* was the only genus significantly more prevalent in saliva from newborns (Cephas et al. 2011). Likewise, in another study from 2011, saliva samples from 74 children (aged 3–18) were ana-

lyzed by pyro-sequencing, and it was demonstrated that the composition of salivary microbiota was altered through transition from deciduous dentition through mixed dentition until permanent dentition, as the proportion of Firmicutes declined, and proportions of Bacteroidetes and Proteobacteria as well as total bacterial diversity increased (Crielaard et al. 2011). Interestingly, recent studies have revealed that the salivary microbiota may be altered in patients with inflammatory bowel disease (Said et al. 2014), which in combination with evidence highlighting dynamic associations between microbial communities in different anatomic sites (Ding and Schloss 2014) suggest that alteration in relation to local and systemic diseases may stress the salivary microbiota, possibly resulting in detectable compositional alterations of the salivary microbiota. Such alterations could potentially be used for identification of disease-prone individuals at early stages of disease.

11.2 Salivary Microbiota in Subjects with Periodontitis

Since the introduction of the bacterial complex theory by Socransky and coworkers (1998), much literature has focused on the etiological association of specific putative periodontal pathogens, i.e., *Porphyromonas gingivalis*, *Prevotella intermedia, Tannerella forsythia* (previously *Tannerella forsythensis*), *Treponema denticola*, *Fusobacterium nucleatum*, and *Campylobacter rectus* in chronic periodontitis and *Aggregatibacter actinomycetemcomitans* in aggressive periodontitis. Thus, several papers have addressed the presence of these specific bacteria as part of the salivary microbiota, and high salivary carriage of suggested periodontal pathogens has been reported in subjects with periodontitis, but also in orally healthy individuals. For example, a large Finnish survey (n = 1294) employed a 16S rRNA-based PCR method with species-specific primers and reported that at least 1 of 6 periopathogens (*T. forsythia, T. denticola, P. gingivalis, C. rectus, A. actinomycetemcomitans* and *P. intermedia*) were identified in 88 % of samples from this adult population, comprised of both periodontitis subjects and orally healthy individuals (Könönen et al. 2007). Likewise, another study employed 16S rRNA-based PCR for examining the presence of 6 periodontal pathogens (*Prevotella nigrescens, A. actinomycetemcomitans, P. gingivalis, T. forsythia, P. intermedia*, and *T. denticola)* in saliva samples from 41 orally healthy children (6–13 years) and reported high carriage in saliva from healthy children of *P. nigrescens* (80 %), *T. denticola* (32 %), *A. actinomycetemcomitans* (24 %), and *P. gingivalis* (12 %) (Kulekci et al. 2008). Moreover, high carriage of suggested periodontal pathogens was also reported in a group of one-year-old children, regardless of whether they were delivered premature and with low birth weight or they were full-term infants (Merglova et al. 2014). Finally, another study using a 16S rRNA-based PCR identification of 4 periodontal pathogens (*A. actinomycetemcomitans, P. gingivalis, F. nucleatum*, and *T. denticola*) in children with and without Fanconi's anemia reported frequent detection of all 4 pathogens in saliva samples, with no significant differences between healthy and diseased children (Lyko et al. 2013). Collectively these reports demonstrate that suggested periopathogens may be present as commensal bacteria in the salivary microbiota. However, several studies have reported that the salivary microbiota may be altered in subjects with periodontitis (Feng et al. 2014; Liljestrand et al. 2014; He et al. 2012; Paju et al. 2009). For example, one study analyzing saliva samples from 1198 Finnish adults showed that presence of two or more suggested periodontal pathogens in saliva was associated with periodontitis as determined by number of teeth with deepened periodontal pockets (Paju et al. 2009). In line, an investigation using quantitative real-time PCR (qRT-PCR) for detection of 4 periodontal pathogens reported significant association between salivary carriage of *P. gingivalis* and *P. intermedia* and chronic periodontitis compared to healthy controls (He et al. 2012). This finding was confirmed in a recent report, as presence of 8 periopathogens were found to be higher in saliva samples from Chinese adults with

chronic and aggressive periodontitis compared to healthy controls (Feng et al. 2014). Interestingly, a study from 2014 demonstrated that high salivary carriage of *P. gingivalis* was associated with increased saliva and serum levels of *P. gingivalis-specific* IgA and IgG, and it was proposed that combined information about saliva carriage of *P. gingivalis* and markers of pathogen-specific host responses could be used as a biomarker for identification of periodontitis patients (Liljestrand et al. 2014). This finding is in line with another study demonstrating that salivary carriage of *P. gingivalis,* in combination with salivary levels of matrix metalloproteinase (MMP)-8 and interleukin 1-β (IL-β), was more strongly associated with periodontitis than one of the tree markers alone (Salminen et al. 2014).

Compositional changes of the salivary microbiota in relation to periodontitis have been investigated in a few studies recently (Yamanaka et al. 2012; Belibasakis et al. 2013; Belstrøm et al. 2014b). In one study, saliva samples from subjects with aggressive periodontitis (n = 21) and chronic periodontitis (n = 20) and saliva samples from healthy control subjects (n = 18) were compared using quantitative fluorescent in situ hybridization (FISH) analysis and demonstrated significantly higher prevalence of *Synergistetes* cluster A in samples from subjects with aggressive and chronic periodontitis. This member of the salivary microbiota might therefore associate with periodontitis (Belibasakis et al. 2013). Additionally, an investigation from 2014, in which 586 saliva samples from an adult Danish population including 139 periodontitis patients and 447 healthy controls were analyzed by means of the HOMIM technology, identified 12 periodontitis-associated phylotypes of saliva. Interestingly, newly suggested periodontal pathogens as *Parvimonas micra* and *Filifactor alocis* were associated with saliva samples from periodontitis subjects, indicating that local bacterial accumulation during periodontitis may shed to, and be identified in, saliva (Belstrøm et al. 2014b). Finally, a recent investigation employed pyro-sequencing of 16S rRNA, for analysis of supragingival plaque samples and saliva samples collected from 19 subjects with periodontitis, before and after periodontal therapy. This study demonstrated that supragingival microbiota differs significantly from salivary microbiota and described that whereas periodontal therapy resulted in a major shift in supragingival microbiota, the salivary microbiota was only modestly influenced by periodontal therapy. Therefore, the authors concluded that shifts of the salivary microbiota might not be a strong predictive measurement of periodontal therapy (Yamanaka et al. 2012). Further large-scale interventional studies using advanced molecular methods are warranted, to elucidate whether local bacterial alterations in periodontitis lesions are reflected by detectable compositional changes of the salivary microbiota.

11.3 Salivary Microbiota in Subjects with Dental Caries

The salivary microbiota in relation to dental caries has been addressed in multiple studies primarily in children (Nurelhuda et al. 2010; Ling et al. 2010; Luo et al. 2012; Chaffee et al. 2014; Relvas et al. 2014) but also in adult populations (Yang et al. 2012; Wennerholm and Emilson 2013; Belstrøm et al. 2014a). Based on their prominent position in the specific plaque hypothesis and their suggested role of true caries pathogens (Parisotto et al. 2010), salivary levels of *Streptococcus mutans* and *Lactobacillus* species in relation to dental caries have been investigated using culture-based methods and different molecular techniques (Guo and Shi 2013). Collectively, *S. mutans* and *Lactobacillus* have been reported to be present in saliva from children and adolescents with dental caries as well as in orally healthy subjects. For example, one study reported that 47 % and 57 % of a population of adolescent had detectable amounts of *S. mutans* and lactobacilli in saliva, respectively, and that 22 % and 34 % of the population had $>10^3$ CFU/ml of these bacteria in saliva (Relvas et al. 2014). In another study, saliva samples were collected from 243 mothers and their newborn infants until 24 months postpartum, and presence of *S. mutans*

and lactobacilli in saliva samples from the mothers was compared to caries incidence in the children at age 3. The study demonstrated that high levels of salivary carriage of *S. mutans* and lactobacilli in mothers were associated with a cumulative incidence ratio of 1.9 of the children having dental caries at age 3. Furthermore, it was demonstrated that children of mothers with high salivary carriage of *S. mutans* and lactobacilli were more likely to be *S. mutans*- and *Lactobacillus-positive* themselves (Chaffee et al. 2014). Likewise, another report suggested that a commercialized test facilitating semiquantitative measurements of salivary *S. mutans* levels could be used for caries risk assessment (Wennerholm and Emilson 2013). Furthermore, recent studies have revealed that the use of probiotics (Cannon et al. 2013) and meticulous oral hygiene instruction (Liu et al. 2014) may result in lowered proportions of *S. mutans* in saliva of children. It has also been reported that salivary levels of *S. mutans* may be altered during different orthodontic procedures (Ortu et al. 2014; Jung et al. 2014), and it has been reported that children with allergic rhinitis have higher salivary levels of *S. mutans* than healthy children (Wongkamhaeng et al. 2014), which is why salivary levels of *S. mutans* may be altered as a consequence of other parameters than dental caries.

Other papers have addressed community profiles of saliva microbiota using advanced molecular methods and demonstrated that dental caries in children and adults associates with an altered salivary microbiota. Collectively, these studies suggest that alterations of saliva microbiota may be more profound than hitherto anticipated based on data from studies only analyzing *S. mutans* and lactobacilli levels of saliva. Thus, alterations in richness and diversity of the salivary microbiota in samples from subjects with dental caries have been reported, and other members of the salivary microbiota have been suggested to be associated with saliva samples from subjects with dental caries. In one study, saliva and supragingival plaque samples from 3- to 6-year-old children with and without dental caries were investigated using high-throughput barcoded pyro-sequencing and PCR-denaturating grading gel electrophoresis for analysis of bacterial diversity and reported identification of 156 genera belonging to 10 phyla in saliva samples from this cohort. Furthermore, salivary microbiota was found to significantly differentiate from microbiota of supragingival plaque. However, no genera identified were found to be significantly associated with saliva samples from subjects with dental caries (Ling et al. 2010). Another study, examining saliva samples from 6- to 8-year-old Chinese children (caries-active n = 30, healthy controls n = 20) using the HOMIM technique, identified 94 bacterial phylotypes representing 30 genera belonging to 6 phyla, out of which 8 and 6 phylotypes were significantly associated with saliva samples from subjects with dental caries and healthy controls, respectively (Luo et al. 2012). A comprehensive report employing 16S rRNA gene amplicon-based and whole-genome-based deep sequencing technologies for analysis of saliva microbiota in samples from 19 caries-active and 26 healthy control subjects (aged 18–22), also reported saliva microbiota in subjects with dental caries to be significantly more diverse than observed in healthy control samples. In addition, 147 caries-associated operational taxonomic units (OTUs) were identified (Yang et al. 2012). Likewise, a study from 2014 investigating saliva samples from 621 adult Danes (caries-active n = 174, healthy controls n = 447) by means of the HOMIM technology found the diversity of salivary microbiota in subjects with dental caries to be reduced compared to healthy controls and identified 10 bacterial phylotypes to be present at different levels in samples from subjects with and without dental caries (5 caries associated and 5 health associated) (Belstrøm et al. 2014a). Finally, in a recent investigation, functional gene signatures were addressed in saliva samples from 10 subjects with dental caries and 10 healthy controls (aged 18–23 years) using a functional gene microarray (HuMiChip 1.0), and it was demonstrated that disease-associated functional gene signatures were associated with saliva samples from subjects with dental caries (Yang et al. 2014). Thus, current evidence suggests that not only may the composition of salivary microbiota be altered in subjects with dental caries but

potentially also the functional abilities of the microbiota may be different in saliva from subjects with dental caries compared to oral health.

11.4 Methodological Considerations for Studying Salivary Microbiota

Some reports describing the composition of the salivary microbiota have been rather conflicting. One explanation might be that different culture-based techniques and molecular techniques were employed, making comparison between investigations difficult. Thus, several reports have demonstrated that meticulous collection and zealous handing of saliva samples in a standardized manner is required for obtaining solid validated results, which can be compared across publications (Lazarevic et al. 2010, 2012, 2013a; Rasiah et al. 2005). Also, it has been reported that use of systemic antibiotics in children with acute otitis media (Lazarevic et al. 2013b), and chemotherapy/radiation therapy in subjects with nasopharyngeal carcinoma (Xu et al. 2014) altered the salivary microbiota. Thus, careful consideration of inclusion and exclusion criteria is prerequisite when analyzing salivary microbiota. Furthermore, previous reports has revealed that application of various DNA extraction protocols (Lazarevic et al. 2013a) and culture-independent molecular techniques have profound influence on the data obtained (Lazarevic et al. 2012). Finally, in a study from 2005 using denaturating gradient gel electrophoresis (DGGE) for analysis of saliva samples, it was suggested that saliva microbiota remains stable for years (Rasiah et al. 2005). In contrast, a recent study from 2010 addressed inter- and intraindividual variations of saliva microbiota by means of pyro-sequencing, and it was concluded that the salivary microbiota cannot be considered stable for more than 5 consecutive days (Lazarevic et al. 2010). This example illustrates that as a consequence of technological improvement, additional methodological considerations must be addressed.

11.5 Future Perspectives: Using the Salivary Microbiota as a Biomarker of Health and Disease

Microbial analysis of supra- and subgingival plaque samples can be tenacious and expensive, since separate sampling and analysis from multiple diseased sites is often required (Yoshizawa et al. 2013). On the other hand, the use of saliva is considerably more cost-effective since only one sample from each subject may be collected and analyzed, thereby making saliva an ideal fluid for analysis of larger populations, which may be suitable for chairside analysis in the dental office (Giannobile et al. 2011; Schafer et al. 2014). Thus, saliva has been proposed as "the mirror of the body," and much scientific activity has focused on identification of salivary biomarkers in relation to oral health and disease (Giannobile et al. 2011). However, it is important to acknowledge that the most common oral diseases, e.g., periodontitis and dental caries, are conditions with multifactorial etiologies, which stresses the importance for combined information from areas of genomics and transcriptomics for identification of disease-prone individuals (Ai et al. 2012). Thus, with further technological developments, it is reasonable to believe that future chairside analysis of saliva samples measuring compositional changes of the salivary microbiota in combination with inflammatory biomarkers could provide diagnostic aid for general practicing dentists, enabling identification of periodontitis and dental caries-prone individuals at early stages of disease, thereby facilitating individualized noninvasive treatment (Yoshizawa et al. 2013).

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Part IV

Future Perspectives in Management of Oral Infections

Use of Probiotics in Future Prevention and Treatment of Oral Infections

12

Mette Rose Jørgensen and Mette Kirstine Keller

Abstract

The interest for probiotic bacteria to combat biofilm-related diseases in the oral cavity has arisen during the last decade. The probiotic concept has successfully been used to control gastrointestinal diseases, such as antibiotic-associated diarrhoea and acute infectious diarrhoea. The potential mechanisms of action in the oral cavity are not fully understood, but there are thoughts to be a local effect in the mouth and systemic effect through immune regulation. The purpose of this chapter is to summarise our current knowledge of the potential for probiotics to improve oral and dental health and to discuss its potential in the future prevention of oral diseases. Despite some limitations, the currently available clinical studies appear expectant, and there seem to be evidence of probiotics to improve oral conditions such as dental caries, periodontitis and the colonisation of *Candida*. However, more long-term clinical studies are needed before evidence-based guidelines can be released.

12.1 Introduction

Probiotics in the dental practice is an emerging and promising strategy to combat biofilm-related diseases in the oral cavity (Laleman and Teughels 2015). The term probiotic derives from the Greek language meaning *pro* "for" -biotic "life". Probiotic bacteria are defined as "Live microorganisms, which when administered in adequate amounts, confer a health benefit on the host" (WHO) (Meurman and Stamatova 2007). Probiotics used in general health should be non-pathogenic and non-toxic, and they should be able to survive in the gastrointestinal tract and temporarily colonise in the gut.

The use of microorganisms to promote health can be dated back to ancient times, where Romans fermented food with microorganisms and used it therapeutically. In 1906, the French physician Henry Tissier isolated the bacteria *Bacillus bifidus communes*, later reclassified as

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the genus *Bifidobacterium*, and some years later, he treated diarrhoea with stool transplants containing bifidobacteria. In 1907, the Nobel Prize–winning scientist Elie Metchnikoff postulated that Bulgarians lived longer because of their consumption of Bulgarian yoghurt (containing lactic acid bacteria) promoting good health. The word "probiotic" was introduced in 1965 by Lilly and Stillwell and probiotics were defined as "substances produced by microorganisms that stimulate the growth of another" (Lilly and Stillwell 1965).

In present time, the probiotic concept has successfully been used to control gastrointestinal diseases, and probiotics also seem to alleviate symptoms of allergy and diseases with immunological pathology (Meurman 2005). The most fully documented probiotic intervention is the treatment of acute infectious diarrhoea, antibiotic-associated diarrhoea and inflammatory bowel disease (Crohn's disease and ulcerative colitis). Furthermore, probiotics are also commonly used for conditions in which firm evidence is lacking such as vaginal candidiasis, *Helicobacter pylori* infection of the stomach and upper respiratory infections (Saha et al. 2012).

There are a number of different microorganisms that can be classified as probiotics, including bacteria and yeasts. The most common probiotic strains belong to the genera *Lactobacillus* (e.g. *L. acidophilus*, *L. rhamnosus*, *L. bulgaricus*, *L. reuteri* and L. *casei*) and *Bifidobacteria* (e.g. *B. bifidum*, *B. longum* and *B. infantis*), but also, some *Streptococcus* spp. (e.g. *S. thermophilus* and *S. salivarius*) and *Saccharomyces* spp. (e.g. *S. boulardii* and *S. cerevisiae*) have been introduced as potential probiotics (Isolauri et al. 2002). *Prebiotics* are defined as non-digestible food ingredients that stimulate selected beneficial bacteria already established in the colon and thus in effect improve host health (Table 12.1) (Dahlén et al. 2012). The most commonly used prebiotics are carbohydrate substrates, e.g. oligosaccharides, with the ability to promote the components of the normal intestinal microflora. When the prebiotics and probiotics are applied together, the concept is defined as synbiotics (Anusha et al. 2015).

Table 12.1 General definitions within probiotic research

Resident microbiota	Consists of the *common* microflora (microorganisms found in most humans), the *supplemental* microflora (microorganisms characterising the individual) and the *transient* microflora (microorganisms temporarily present in the body)
Probiotic	Living microorganisms which, when ingested, provide a health benefit on the host
Prebiotic	Non-digestible food ingredients, e.g. oligosaccharides, inulin and lactulose, that stimulate the growth and/or activity of selected beneficial bacteria
Synbiotic	Nutritional supplements combining probiotics and prebiotics synergistically, which beneficially affect the host

During the last decade, the interest in probiotics as an alternative, preventive, and therapeutic approach in the oral cavity has arisen. The efficacy of probiotic bacteria in the oral cavity has been investigated for conditions including dental caries, gingivitis, periodontitis, halitosis, colonisation of oral *Candida*, oral mucositis and xerostomia. Within dentistry, *L. rhamnosus GG* and *L. reuteri* are the most intensely studied probiotic species, and they have shown their potential in interacting with *S. mutans* and reducing colonisation of *Candida*. The intention of this chapter is to briefly outline current knowledge on the potential for use of probiotic bacteria to prevent oral diseases and to improve oral and dental health.

12.2 Mechanisms of Action

The mechanisms of action of probiotics are not fully understood but are thought to be locally in the mouth by competing for adhesion sites and nutrients with the oral pathogens and by inhibition of growth of pathogens by production of bacteriocins or other products (acid or peroxide). Thus, probiotics modify the composition of the oral biofilm or the metabolic activity. There also seems to be a systemic regulation of the immune response during intake of probiotics (Devine and Marsh 2009; Stamatova and

Meurman 2009). Alterations in levels of both salivary IgA (Ericson et al. 2013) and cytokines in the gingival crevicular fluid (Twetman et al. 2009) have been registered after exposure to probiotic bacteria. Generally, the effects of probiotic bacteria are strain specific and cannot be applied directly to other strains. Also, the same strains can have different effect in different individuals (Koll-Klais et al. 2005).

12.3 Probiotics and Oral Diseases

12.3.1 Dental Caries

Dental caries is demineralisation of the tooth induced by microbial production of acid in the dental plaque. Hence, modulation of the dental plaque by probiotic bacteria has naturally been of interest.

Since the first study on the topic by Näse et al. in 2001, there have been an increasing number of clinical trials with caries-related end points. However, the majority of these studies have other end points than caries such as microbial counts or plaque index. Mutans streptococci are the most common microbial end point, but some studies also look at lactobacilli. Most of these studies show an inhibitory effect of probiotics on salivary mutans streptococci, but a few does not find any difference after the intervention period (Stecksen-Blicks et al. 2009; Lexner et al. 2010; Taipale et al. 2012). Nonetheless, two recent systematic reviews conclude that probiotic bacteria decrease the mutans streptococci counts as long as there is a regular intake of the probiotic bacteria (Cagetti et al. 2013; Laleman et al. 2014). The strains used in the clinical intervention trials vary among different lactobacilli strains and a few different bifidobacteria and even some streptococci strains. There does not seem to be any clear-cut difference between the outcomes of the trials based on the strains used in the study.

Since the lactobacilli used in most studies have strong acidogenic abilities, a natural reservation in relation to caries would be that addition of lactobacilli to the oral cavity would involve an increased risk of an acidogenic shift of the oral microbiota. However, two clinical trials found no increase in biofilm acidurity after exposure to probiotic lactobacilli strains (*L. reuteri* SD2112, *L. reuteri* DSM 17938 and *L. reuteri* PTA 5289) (Keller and Twetman 2012; Marttinen et al. 2012).

There are fewer studies with caries incidence, root caries arrest or remineralisation of carious lesions as outcome. The six studies with caries incidence as end points are listed in Fig. 12.1, which displays the prevented fraction of the studies. The calculated mean of preventive fraction is 33 %. The most popular vehicle for the probiotic bacteria was milk which was supplemented with 2.5 ppm fluoride in one study. The first study from Finland (Näse et al. 2001) also used milk as the vehicle for *L. rhamnosus* GG during a

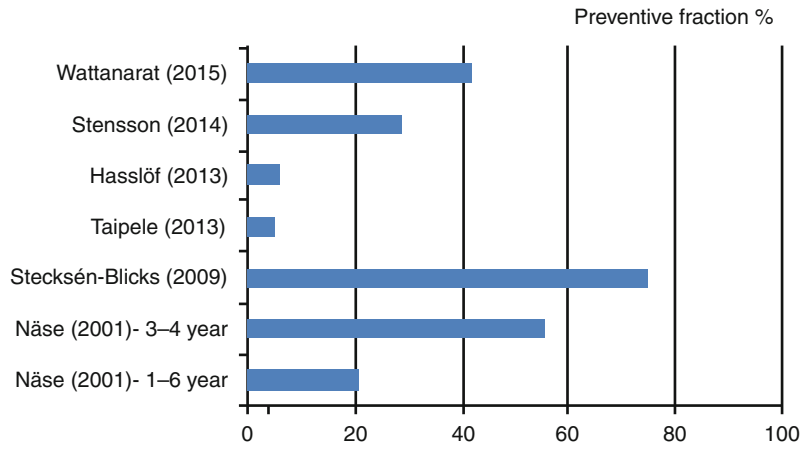

Fig. 12.1 Preventive fractions in clinical studies with caries as an end point

7-month intervention period. They found a preventive fraction of 56 % among the children being 3–4 years of age but only 21 % when looking at the entire study population of 1–6 years of age. Besides the dental health benefits, the study also showed a reduction in antibiotic treatments for respiratory infections in the test group (Hatakka et al. 2001). The promising results encouraged a similar study in Sweden where preschoolers aged 1–5 years were provided with milk containing *L. rhamnosus* LB21 and 2.5 ppm fluoride on a daily basis (Stecksen-Blicks et al. 2009). Their results showed a 75 % preventive fraction but due to the study design, it was not possible to determine how much of this can be attributed to the fluoridation or the addition of probiotic bacteria. Secondly, this study also found an additional beneficial effect in fewer prescriptions of antibiotics to the children in the test group. A more recent study from Finland chose another vehicle based on the young age of the participants (0–12 months) and used a pacifier with room for probiotic tablets to obtain longer oral exposure to the probiotic strain *L. rhamnosus* LB21 (Taipale et al. 2013). At age four, however, the caries preventive fraction was rather modest (5 %) which might be due to uncertain compliance.

Two Swedish studies are follow-up studies on trials conducted 6 and 7 years ago, respectively. After having eaten gruel with added *L. paracasei* F19 during infancy, the children in the test group had statistically significant less eczema at age 6 but not statistical significant results on caries incidence (Hasslof et al. 2013). The dropout rate was rather high and together with generally low level of caries, it may account for failure to achieve statistically significant results. In comparison, the results of the other study were more promising. Stensson et al. (2014) reports a preventive fraction of 29 % 7 years after the original intervention with five drops of *L. reuteri* ATCC a day during the first 12 months of age.

It has been discussed whether colonisation of the probiotic strains is necessary to gain an effect. The studies with microbial end points show a change in levels of mutans streptococci as long as the probiotic strains are administered but mutans streptococci return to previous levels when the probiotic bacteria are ceased. Hence, the effect seems to be dependent of a continuous distribution of the probiotic bacteria. However, the follow-up study by Stensson reported on an effect 7 years after the original intervention. This could point towards the importance of intervention in early childhood to secure a long-term effect. Despite the promising results from the existing studies, there is still insufficient evidence to give any clinical recommendations, and more long-term studies with caries as an end point are needed.

12.4 Gingivitis and Periodontitis

Gingivitis and periodontitis are diseases related to the gum and surrounding bone caused by bacterial dental plaque and the host immune response. Exposure of the gingival tissues to dental plaque results in inflammation within the tissues which manifests in clinical signs of gingivitis, e.g. change in colour, swelling of the tissue, increased gingival exudate and bleeding upon provocation. The clinical features of chronic periodontitis are, in addition to the characteristics of gingivitis, loss of clinical attachment and loss of alveolar bone. Today, treatment strategies include hygiene improvement, mechanical debridement (scaling and root planing), surgery and antibiotic treatment. One of the main etiological factors in periodontal inflammation is the shift of the periodontal microbiota towards gram-negative species and the absence of so-called beneficial bacteria. Theoretically, restoring the reduced number of these beneficial bacteria via probiotic administration might be of interest in the treatment of periodontal disease (Teughels et al. 2008).

A Medline search in June 2015 revealed 24 *in vivo* human clinical studies concerning the effect of probiotic bacteria on periodontally healthy individuals or patients with gingivitis or periodontitis. Selected studies are shown in Table 12.2. These studies mostly report end points related to amount of plaque (PI), gingival condition (GI), bleeding on probing (BOP), probing pocket depth (PPD) and subgingival

Table 12.2 Human clinical studies with outcome related to periodontal disease

Infection type at baseline	Reference	Strain	Vehicle, time	Assessment criteria	Effect of probiotic treatment
Healthy volunteers	Kang et al. (2006)	*W. cibaria* CSM1	Rinse, 1 day	OHI-S, PI	Yes
	Shimauchi et al. (2008)	*L. salivarius* WB21	Tablets, 8 weeks	PI, GI, BOP	Yes
	Iwamoto et al. (2010)	*L. salivarius* WB21	Tablet, 4 weeks	BOP, PPD	Yes
	Sinkiewicz et al. (2010)	*L. reuteri* ATCC 55730/ATCC PTA 5289	Diet, 12 weeks	PI, periopath.	Yes
Gingivitis	Krasse et al. (2006)	*L. reuteri*	Chewing gum, 2 weeks	GI, PI	Yes
	Twetman et al. (2009)	*L. reuteri* ATCC 55730/ATCC PTA 5289	Chewing gum, 2 weeks	BOP, GCF, cytokines	Yes
Exp.	Staab et al. (2009)	*L. casei Shirota*	Milk drink, 8 weeks	PI, GI, GCF	Yes
Exp.	Slawik et al. (2011)	*L. casei Shirota*	Milk drink, 4 weeks	PI, BOP	Yes
	Iniesta et al. (2012)	*L. reuteri* ATCC 55730/ATCC PTA 5289	Tablets, 8 weeks	PI, GI	No
Exp.	Hallström et al. (2013)	*L. reuteri* ATCC 55730/ATCC PTA 5289	Tablets, 3 weeks	PI, GI, BOP	No
Periodontitis	Riccia et al. (2007)	*L. brevis*	Lozenges, 4 days	GI, PI, BOP, calculus	Yes
	Tsubura et al. (2009)	*B. subtilis* E-300	Rinse, 30 days	PPD, BOP, GI	No
SRP	Vivekanada et al. (2010)	*L. reuteri* DSM 17938/ATCC PTA 5289	Lozenges, 3 weeks	PI, GI, BOP, PPD, CAL	Yes
	Vicario et al. (2013)	*L. reuteri* ATCC 55730/ATCC PTA 5289	Tablets, 4 weeks	PI, BOP, PPD	Yes
SRP	Teughels et al. (2013)	*L. reuteri* DSM 17938/ATCC PTA 5289	Tablets, 12 weeks	PPD, CAL, BOP, periopath.	Yes
SRP	Shah et al. (2013)	*L. brevis*	Lozenges, 2 weeks	GI, PI, PPD, CAL	Yes
	Szkaradkiewicz et al. (2014)	*L. reuteri* ATCC PTA 5289	Tablets, 2 weeks	GI, PPD, CAL	Yes
	Tekce et al. (2015)	*L. reuteri* DSM 17938/ATCC PTA 5289	Lozenges, 3 weeks	PI, GI, BOP, PPD	Yes

Exp. experimental gingivitis, *SRP* scaling and root planing, *OHI-S* oral hygiene index, *PI* plaque index, *GI* gingival index, *BOP* bleeding on probing, *PPD* probing pocket depth, *CAL* clinical attachment level, *GCF* gingival crevicular fluid, *periopath.* periopathogens

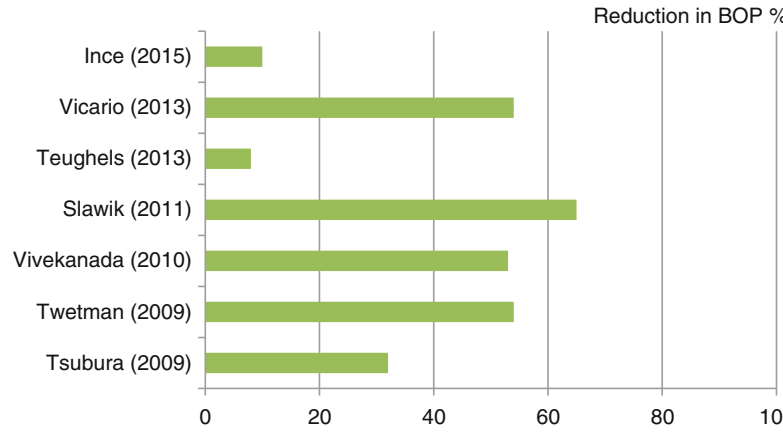

Fig. 12.2 Percent reduction in BOP between probiotic and placebo groups

microbiota associated with periodontal diseases. In the majority of the studies, a significant effect of probiotic treatment was obtained in the probiotic group compared to placebo. However, the studies are heterogeneous and have been subject to methodological criticism mainly due to a diverse patient population, lack of descriptions of the extent and severity of the periodontal disease, potential confounding factors, high risk of bias and inconsistent end points (Dhingra 2012; Laleman and Teughels 2015).

Eight clinical studies have looked at clinical periodontal parameters in periodontally healthy individuals (Burton et al. 2013; Iwamoto et al. 2010; Kang et al. 2006; Karuppaiah et al. 2013; Mayanagi et al. 2009; Shimauchi et al. 2008; Sinkiewicz et al. 2010; Zahradnik et al. 2009). In a randomised, double-blind, placebo-controlled trial, Shimauchi et al. (2008) investigated the effect of *L. salivarius* WB21 on 66 healthy subjects and found periodontal parameters improved after 8-week intervention. Mayanagi et al. (2009) and Zahradnik et al. (2009) found a reduction in selected periopathogens (e.g. *P. gingivalis*) in subgingival plaque and saliva of healthy individuals after treatment with lactobacilli spp. Two randomised clinical trials have looked into the efficacy of probiotics on gingival health in children. In the first study by Burton and co-workers (2013), 100 children (5–10 years) were included to assess changes in plaque score and gingival score after 3 months treatment with either *S. salivarius* M18 or placebo. At treatment end, the plaque scores were significantly lower in the probiotic group, but no differences were seen in gingival scores. These findings were confirmed by Karuppaiah et al. (2013).

Krasse et al. (2006) were the first to investigate the effect of chewing gum containing *L. reuteri* on patients with chronic gingivitis. They found a reduction in PI and GI after 2-week intervention. However, Iniesta and co-workers (2012) were not able to confirm these findings. Three clinical trials have looked upon the effect of probiotics on subjects with experimental gingivitis (Hallström et al. 2013; Slawik et al. 2011; Staab et al. 2009): two with positive and one with a negative outcome (Table 12.2).

Bleeding on probing (BOP) is a widely used criterion to diagnose gingival inflammation. Seven studies found decreased BOP after treatment with probiotic bacteria compared with placebo (Fig. 12.2) (Ince et al. 2015; Slawik et al. 2011; Teughels et al. 2013; Tsubura et al. 2009; Twetman et al. 2009; Vicario et al. 2013; Vivekananda et al. 2010). However, Teughels et al. (2013) did not find this decrease significant.

Nine recent studies (2007–2015) have investigated the effect of probiotics on patients with chronic periodontitis (Ince et al. 2015; Riccia et al. 2007; Shah et al. 2013; Szkaradkiewicz et al. 2014; Tekce et al. 2015; Teughels et al. 2013; Tsubura et al. 2009; Vicario et al. 2013; Vivekananda et al. 2010) (Table 12.2). Longitudinal studies have shown the efficacy of the standard treatment approach consisting of systematic scaling and root

planing (SRP) on root surfaces and optimal oral hygiene. Three studies combined the SRP with probiotic treatment. In a randomised placebo-controlled trial from 2013, Teughels et al. proved the effect of *L. reuteri* (Prodentis)-containing probiotic lozenges as an adjunct to SRP in patients with chronic periodontitis. Significantly larger PPD reductions, especially in moderate and deep pockets, were evident.

Antibiotic treatment can be an adjunct to mechanical therapy when these are found to be insufficient. However, repeated use of antibiotics increases the risk of drug-resistant microorganisms. Shah et al. (2013) compared the efficacy of probiotic tablets (*L. brevis* CD2) alone, in combination with doxycycline and doxycycline alone after SRP in patients with aggressive periodontitis. The study showed that all three alternatives had similar reducing effect on PI, GI and PPD after 2 months. This finding is encouraging since a reduction in the use of antibiotics in the dental practice is desirable.

In summary, within the limitations of the clinical studies performed to date, the results are encouraging and display that probiotics might be a valid supplement to the gold standard treatment of gingivitis and chronic periodontitis patients. However, further long-term studies with more homogenous end points are needed before evidence-based treatment recommendations can be released.

12.4.1 Colonisation of Oral *Candida* Species

The yeast *Candida* can cause a number of disorders in the oral cavity, known as oral candidiasis. Seven *Candida* species are the clinically most important, of which *C. albicans*, *C. tropicalis* and *C. glabrata* are most frequently isolated (80 %). *Candida* species are commensal microorganisms in the oral cavity in 40–60 % of the population and only cause disease when disturbances in the oral microbial balance occur (Teughels et al. 2008). Oral candidiasis is therefore often seen in elderly people and is frequently associated with antibiotic treatment, hyposalivation, impaired local or systemic immune system, neglected oral hygiene, dentures and smoking (Anil et al. 2014; Pires et al. 2002; Shay et al. 1997; Torres et al. 2002). A number of antifungal agents are available for the treatment of oral candidiasis, e.g. the polyenes (nystatin) and the azoles (fluconazole). However, since oral candidiasis is caused by an ecological imbalance (dysbiosis) in the oral biofilm that favours fungal overgrowth, a certain interest has been addressed to a bioecological approach for prevention and management.

In the past decade, several clinical studies have investigated the ability of probiotic bacteria to hamper the growth of *Candida* in the oral cavity. Hatakka and co-workers (2007) were the first to conduct a randomised double-blind, placebo-controlled trial on the effect of probiotics on the prevalence of oral *Candida*. The study included 276 elderly people consuming probiotic cheese (*L. rhamnosus GG*, *L. rhamnosus LC705*, *Propionibacterium freudenreichii spp. shermanii JS*) or placebo cheese for 16 weeks. The investigators found that the prevalence of high yeast count ($\geq 10^4$ colony-forming units (cfu)/mL saliva) diminished by 32 % in the probiotic cheese group, while it increased by 21 % in the control group. These findings were confirmed in two later studies following consumption of yoghurt containing lactobacilli and bifidobacteria spp. (Dos Santos et al. 2009; Mendonca et al. 2012). The probiotic concept has also been proven effective in reducing the number of *Candida* species in patients with *Candida-associated* stomatitis (Li et al. 2014) and in candidiasis-asymptomatic elderly denture wearers (Ishikawa et al. 2015).

Our knowledge within this field was just recently confirmed by Kraft-Bodi and co-workers (2015) who investigated the effect of a twice daily intake of probiotic lactobacilli lozenges on the prevalence and counts of oral *Candida* species in 215 frail elderly living in nursery homes in the southern parts of Sweden. The results revealed a significant reduction of high *Candida* counts ($>10^4$ cfu/mL) in saliva in the test group compared to placebo after 12-week treatment with *L. reuteri* (DSM 17938 and ATCC PTA 5289) (Fig. 12.3). The same results were seen regarding *Candida* prevalence in plaque after probiotic treatment.

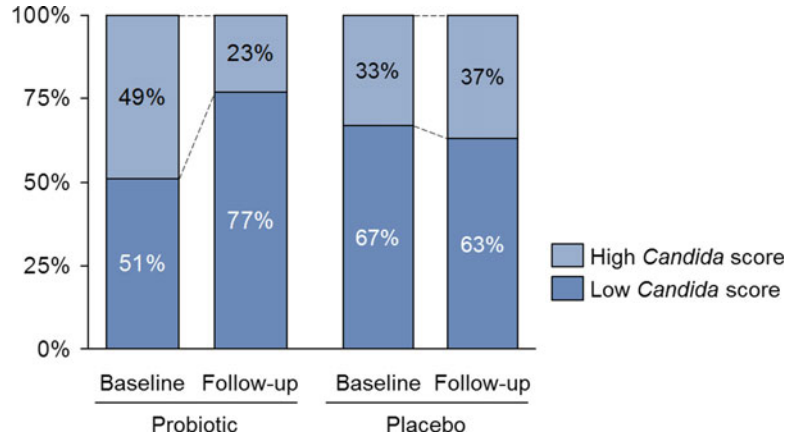

Fig. 12.3 Percent distribution of *Candida* scores in saliva at baseline and at follow-up in the probiotic and placebo groups

In conclusion, our knowledge from the findings of these studies suggest that probiotic bacteria added to food, tablets or lozenges may reduce oral *Candida* counts, and we thereby might be one step closer to clinical recommendations on the use of daily probiotic supplementation in patients at risk for oral candidiasis.

12.5 Oral Mucositis

Oral mucositis is a frequent and painful side effect of cancer chemotherapy and radiotherapy to cancer in the head and neck region. The condition can result in suboptimal oral hygiene, problems with adequate nutritional intake and reduced quality of life. The immunosuppressed patients have earlier been mentioned as a group where probiotic bacteria should be avoided or used only with caution. However, a randomised, controlled trial with 200 patients found no serious adverse effects of the probiotic product (Sharma et al. 2012). Furthermore, they found a statistically significant reduction in the prevalence of mucositis (7 % vs. 28 %) and reduction of the proportion of patients with severe (grades III and IV) mucositis (52 % vs. 77 %).

12.6 Halitosis

Oral malodour is often caused by compounds from the oral cavity but some compounds may also be produced by nasal, oropharyngeal or pharyngeal infections (Delanghe et al. 1997; Quirynen et al. 2009). The oral malodours can be produced by anaerobic bacteria by degradation of food residues, exfoliated epithelial cells and salivary proteins (Scully and Greenman 2008). It has been proposed that bacteriotherapy in order to replace halitosis-associated species may create a more long-lasting effect than the current methods reducing the total bacterial numbers by mechanical measures such as brushing, flossing, and tongue scraping, antibacterial agents, and complex binding of malodorous compounds by zinc (Burton et al. 2005; Fedorowicz et al. 2008; Outhouse et al. 2006; Porter and Scully 2006). Several studies have looked at the effect of probiotic bacteria on oral malodour (Burton et al. 2006; Iwamoto et al. 2010; Kang et al. 2006; Keller et al. 2012; Sutula et al. 2013; Suzuki et al. 2014). A number of them find a reduction in either VSC (volatile sulphur components) or organoleptic score. However, they all have fairly small study groups ($n = 22–46$) and some are not placebo controlled.

12.7 Vehicles, Dose and Safety

The safety of administration of probiotic bacteria must also be considered. Overall, the use of probiotics is considered safe. Some strains used as probiotics, also strains used in oral health, have been isolated from infections such as endocarditis (Cannon et al. 2005). However, in both Finland and Sweden where use of probiotic products

containing *Lactobacillus* is common, there have been no increase in *Lactobacillus*-associated bacteremia in a period with increasing use of the strains (Isolauri et al. 2002; Salminen et al. 2004; Sullivan and Nord 2006). In another study, the safety of two strains used to promote gastrointestinal health in infants was evaluated, and no side effects were found (Saavedra et al. 2004). However, in some high-risk groups, adverse effects have been reported (Cilieborg et al. 2011).

So far, no serious adverse effects have been reported in clinical trials regarding oral healthcare. Some of the reported adverse effects include flatulence or mild abdominal discomfort. A few studies have addressed this issue as a primary outcome and no adverse effects have been found either in animal models (Tanzer et al. 2010) or in humans (Burton et al. 2011). The acidogenicity of lactobacilli could lead to increased risk of caries, and hence, acidogenicity (Pham et al. 2009) and sugar fermentation (Haukioja et al. 2008; Hedberg et al. 2008) of probiotic strains have been studied *in vitro*. However, as previously mentioned, no increased plaque acidogenicity has been found *in vivo*.

12.8 Considerations for the Future

The currently available studies regarding the potential for probiotics to improve oral and dental health are promising. The studies display that probiotics might help to improve oral conditions such as dental caries, periodontitis, halitosis, mucositis and oral *Candida* load. Emerging research in human and animal models has also indicated that probiotics may enhance chronic wound healing, which could be beneficial in the oral cavity (Huseini et al. 2012; Jones and Versalovic 2009; Sonal Sekhar et al. 2014). Several health-promoting effects of probiotics are well recognised, but their influence on oral health still needs to be elucidated by more long-term, randomised clinical trials. The potential mechanisms of action of probiotics in the oral cavity are not fully understood but are anticipated to be similar to those observed in the gastrointestinal tract. However, it is important to keep in mind that the oral cavity differs from the gut with regard to oral microbiota, mucosal structure and composition of fluids like saliva (Meurman and Stamatova 2007). Future studies should focus on defining the optimal strains for the various dental diseases. Moreover, more studies are needed to clarify whether a mix of different strains works better than single strains, determine the optimal daily dosage and find a vehicle system allowing a prolonged retention time in the oral cavity.

Conclusion

The beneficial effect of probiotic bacteria has been suggested and studied within several areas of dentistry. Despite limitations of the studies, such as surrogate end points, heterogeneous design and small sample sizes, the majority of the studies show promising results. Still, there is not sufficient evidence to make any clinical recommendations but when for either prevention or treatment of oral infections, probiotic bacteria should be used as an adjunct to the existing options.

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Management of Patients with Oral Candidiasis

13

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Abstract

Oral candidal infections are medically treated with antifungal agents. In the fungal cell membrane, steroid ergosterol is the target of the antifungals on the market, but similarity with the human cell membrane may cause host toxicity and unintended reactions. Management of oral candidiasis depends on several factors, some are host-sensitive parameters, systemic diseases and drug exposure, and others are infection-sensitive parameters, duration of the infection and the virulence of the infecting *Candida* species. Treatment failure might be associated with acquired or native azole resistance in particular in patients with recurrent oral candidiasis. This risk can be reduced if different types of antifungal drugs are used over time or are combined. This chapter focuses on antifungal treatment of the medically compromised patient with oral candidiasis by highlighting the advantages and disadvantages of different antifungals.

13.1 Introduction

Oral candidal infections are medically treated with antifungal agents with different indications. Fungi are eukaryotes like humans, which is challenging for the drug selectivity as similarity between the human host and the pathogen may cause host toxicity and unintended reactions. Human and fungal cell membrane steroids differ, which has been the focus of the antifungals on the market. Human cell membranes consist mainly of cholesterol, whereas fungal cell membranes consist of ergosterol, and ergosterol and its biosynthesis have been the drug target of antifungals (Xie et al. 2014).

Antifungal agents for treatment of oral candidiasis fall into two main categories: polyenes (nystatin and amphotericin B) and azoles (miconazole, clotrimazole, fluconazole, ketoconazole, and itraconazole). Antifungal agents seem to interact with the ability of candidal adherence to buccal epithelium and some are up-concentrated in epithelial cells, e.g. itraconazole. Topical and systemic formulations are available.

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13.2 Polyenes

Nystatin and amphotericin B are ergosterol synthesis inhibitors and broad-spectrum antifungal agents. They interact with ergosterol in the cell membrane of yeasts and yeastlike fungi, thus, all the *Candida* spp. The binding to ergosterol causes pore formation and ion leakage. Depending on the drug concentration on the application site, polyenes have a fungistatic or fungicidal effect. Amphotericin B has not absolute specificity for fungal cell membranes as it also binds to cholesterol of the human cell membranes, which causes significant systemic toxicity.

Thus, polyenes are only available for topical treatment of oral candidiasis. When administered perorally both are poorly absorbed from the oral, gastric, and intestinal mucous membranes and mostly eliminated faecally unchanged. Accordingly, they have no known severe drug-drug interactions. Nystatin has a bad bitter taste and formulations are often sweetened with sucrose, which significantly increases the risk of dental caries. Special formulations without sucrose can be prepared if not available on the market. The adverse drug effects of topical polyenes are usually mild and of gastrointestinal character.

13.3 Azoles

Azoles are cytochrome p450 enzyme inhibitors and the antifungal effect is interaction with the fungal ergosterol synthesis through binding to the cytochrome p450 enzyme, CYP51. A fungistatic effect is achieved by ergosterol depletion, changes in cell membrane permeability and membrane-bound proteins, and synthesis of cell toxic sterols. Some *Candida* spp., i.e. *C. krusei* and *C. glabrata*, are native resistant or have reduced sensibility to azoles (Arendrup 2013). Azoles can enhance the effect of oral antidiabetics leading to hypoglycaemia.

Azoles are available for topical and systemic use. Azoles for oral candidiasis are classified into imidazoles with a two-nitrogen azole ring (clotrimazole and miconazole) and triazoles with a three-nitrogen azole ring (fluconazole and itraconazole). All these azoles are cytochrome p450 inhibitors, i.e. CYP2C9 and CYP3A4. These enzymes are involved in the metabolism of approximately 40 % of the marketed drugs. Thus, azole-drug interaction must be taken into serious consideration in medically compromised patients.

Clotrimazole cream has a low skin penetration and has no clinical relevant drug-drug interactions. Miconazole is a potent CYP2C9 and CYP3A4 inhibitor. Only 25 % of the miconazole administered topically is absorbed from the skin and mucous membranes of the mouth, ventricle, and intestine, but the first-pass intestinal and liver enzymes are inhibited significantly. Miconazole has clinical relevant drug-drug interactions where dose adjustment of the implicated drug is important, e.g. warfarin and cyclosporine. Clotrimazole and miconazole have a bacteriostatic effect that is beneficial in treating angular cheilitis.

Fluconazole is both available as oral suspension for topical/systemic treatment and as capsules for systemic use. The oral suspension has both topical and systemic effects as it is absorbed intestinally and secreted in saliva in high concentration after swallowing. The majority of fluconazole is excreted unchanged by the kidneys, why knowledge of reduced renal function is relevant. If the glomerular filtration rate (GFR) is <50 %, the dosage is reduced with 50 % because of 50 % longer elimination time. Fluconazole is a potent inhibitor of CYP2C9 and a moderate inhibitor of CYP3A4 why there is a risk of drug-drug interaction with a long list of drugs (The American Society of Health-System Pharmacists (ASHP) 2015, The Danish Health and Medicines Authority 2015).

Itraconazole is available for systemic use and is absorbed intestinally. It is only recommended to adults. The absorption is dependent on gastric pH, why it must be administered before a meal, and proton pump inhibitors reduce the absorption. Itraconazole is metabolized in the liver and is a potent CYP3A4 inhibitor. Like fluconazole, itraconazole interacts with a long list of drugs (The American Society of Health-System Pharmacists (ASHP) 2015, The Danish Health and Medicines Authority 2015).

Ketoconazole is recommended for topical skin infections only. In 2013 the European Medicines Agency (EMA) assessment and the US Food and Drug Administration (FDA) recom-

Table 13.1 Advantages and disadvantages of topical and systemic antifungal treatment of oral candidiasis

Sensitive parameters	Topical		Systemic
	Polyenes	Azoles	Azoles
Difficult compliance	–	–	+
Complicating systemic disease/condition	+	+	–
Risk of drug-drug interactions	+	–	–
Azole insusceptibility	+	–	–
Treatment expense	–	–	+

+ Advantage, – disadvantage

Table 13.2 Relevant factors for management of patents with oral candidiasis

Host-sensitive parameters	
1. Predispositions for oral candidiasis	Local
	Systemic
2. Health and medical status of the patient affecting drug metabolism	Chronic diseases
	Drug exposure
Infection-sensitive parameters	
1. Classification of infection	Duration of infection (acute/chronic)
	Primary/secondary/tertiary infection
	Sporadic/recurrent
	Clinical manifestations
2. *Candida* species	Susceptibility to antifungals
	Resistance to antifungals

mended that ketoconazole was no longer used for systemic treatment of candidiasis because of high risk of severe adverse reactions, i.e. liver damage, drug interactions, and adrenal gland problems (European Medicines Agency 2013; US Food and Drug Administration 2013).

The adverse drug effects of azoles are usually mild and of gastrointestinal character. Topical preparations may give rise to local skin irritation.

The treatment expense for different antifungals and different formulations may vary considerably and must also be taken into account in treatment planning. The general advantages and disadvantages of topical and systemic treatment are listed in Table 13.1.

13.4 Specialist Treatment

Second-generation azoles such as voriconazole and posaconazole are spared for specialist settings and selected patients with life-threatening candidiasis or severe immunocompromising conditions. Intravenous antifungal therapy with amphotericin B, echinocandins, and pyrimidines for oral candidiasis may be indicated in seriously medically compromised patients and should only be done in specialist settings.

13.5 Management of Patents with Oral Candidiasis

Management of oral candidiasis depends on several factors; some are host-sensitive and others are infection-sensitive parameters (Table 13.2).

13.5.1 Host-Sensitive Parameters

Host-dependent parameters are very important factors when managing oral candidiasis. Identification of predisposing factors is crucial for successful treatment outcome (Table 13.3). If the underlying causes are not eliminated or identified, the chance of relapse is high.

The drug formulation is relevant in patients with special needs, e.g. topical formulation in patients with dysphagia and capsules in patients

Table 13.3 Local and systemic predisposing factors and conditions

	Background	Intervention
Local	Poor oral hygiene	Instruction, motivation, and follow-up of oral hygiene procedures
	High-carbohydrate diet	Information, motivation, and follow-up
	Salivary gland hypofunction caused by medications, head and neck radiotherapy, or systemic diseases like Sjögren's syndrome	Stimulation of functional salivary gland tissue with sugar-free pastilles or chewing gum, substitution of drug if possible
	Mucosal traumas	Identify cause and eliminate
	Mucosal diseases	Diagnostic workup, information, treatment, and follow-up
	Topical steroid	Instruction in appropriate behaviour
	Tobacco	Tobacco counselling, smoking cessation
Systemic	Immune deficiency (acquired or idiopathic)	Diagnostic workup and intervention. Often need for multidisciplinary cooperation
	Systemic diseases	
	Nutritional deficiency	

with reduced compliance. Patients with hyposalivation cannot dissolve pastilles and lozenges.

The health and medical status of the patient is important as the choice of antifungals depends on whether the patient can eliminate the drug in order to avoid toxic or adverse reactions. Moreover, knowledge of the patient's drug intake is vital as severe drug-drug interactions with the antifungals can be relevant (Table 13.1 and 13.4).

In treatment of pregnant women, knowledge of teratogenic risks in relation to drug exposure is always important as is unintended drug exposure of breastfed children. Generally, well-known and well-documented drugs are recommended during pregnancy and breastfeeding. Topical nystatin and amphotericin B are well-documented old antifungal agents and have a low systemic effect because of little absorption. Topical miconazole for vaginal candidiasis in pregnant women is widely used without report of higher incidence of foetal malformations. Foetal malformations have been reported in relation to high-dose fluconazole and also itraconazole should be avoided in pregnancy and during periods of breastfeeding.

13.5.2 Infection-Sensitive Parameters

Infection-sensitive parameters influence the duration of treatment as acute infection usually needs a shorter intervention period. Acute candidiasis in general needs topical treatment for 2 weeks and systemic treatment for 1 week, whereas the chronic infection needs longer intervention (Table 13.4). The anatomic area of infection influences the choice of drug vehicle. Cream is used for perioral infections but is not applicable on wet oral mucous membranes. Topical formulations for intraoral infections are pastilles, lozenges, oral gel, and oral suspension. All topical agents need frequent dosage as oral clearance results in falling of the concentration to a subtherapeutic level after approximately 6 hours. To achieve maximal retention time, topical treatment must be applied after meals and oral hygiene procedures, and the patient must also be instructed not to drink and rinse the mouth after application. Thus, topical treatment is challenging for the compliance of the patient.

All lesions in multifocal infections need to be treated; thus, these are most easily treated with systemic administration of the antifungal agent. However, this is not always possible because of other host- or infection-sensitive parameters, e.g. azole-drug interactions (Table 13.1).

Some of the *Candida* spp. commonly isolated from the oral cavity are not sensitive to azole antifungal agents, e.g. *C. krusei* and *C. glabrata,* and drug resistance can be acquired by other species (Tables 13.1 and 13.4) (Arendrup 2013).

Acquired resistance to polyenes is very rare and accordingly little described. The low

Table 13.4 Central host- and infection-sensitive parameters regarding the management of patients with chronic oral candidiasis with antifungals

				Host factors		Infection factors		
Administration	Drug	Formula	Dosage	Diseases	Drug interaction	Classification	In-sensitive *Candida* species	Comments
Topical	Clotrimazole	Cream 1 %	2–3 daily continues until 10 days after lesions healed	–	–	Perioral *	–	>12 years of old
	Miconazole	Cream 2 %	2 daily continues until 10 days after lesions healed	–	+	Perioral *	+	
	Ketoconazole	Cream 2 %	1–2 daily continues until some days after lesions healed	Liver monitoring	+	Perioral	+	
		Oral gel	2.5 ml × 4 daily in 4–6 weeks	–	+	Intraoral	+	Blockage of airway in neonatal children
	Nystatin	Oral suspension 100,000 IE/ml	1 ml × 4 daily in 4–6 weeks	–	–	Intraoral	–	
		Pastille 100.000 IE/unit	After meals x 4 daily 4 weeks	–	–	Intraoral	–	Not if hyposalivation
	Amphotericin B	Lozenge 10 mg	4 times daily for 4 weeks	–	–	Intraoral	–	Not if hyposalivation
	Fluconazole	Oral suspension 10 mg/ml	50–100 mg daily for 4 weeks	Dosis↓ reduced renal function	+	Multi-focal infection	+	
Systemic	Fluconazole	Tablet or capsule 50–100 mg	1 daily in 2–3 weeks	Dosis↓ reduced renal function	+	Multi-focal infection	+	High compliance
	Itraconazole	Tablet or capsule 100 mg	1 daily in 2 weeks	Not cardiac insufficiency Caution liver disease	+	Multi-focal infection	+	High compliance

–: not relevant
+: relevant
*: some additional antibacterial effect
Indications, drug and formula availability may vary in different countries

incidence is believed to be related to the fungicidal effect of polyenes. The exact mechanisms of resistance are not clear but are suggested to be related to mutations in the ergosterol biosynthesis pathway (Maubon et al. 2014). On the contrary, acquired resistance towards azoles is widely described and believed to be related to their fungistatic effect.

There are four main azole resistance mechanisms:

1. Reduced affinity to drug target CYP51
2. Increased amount of drug target CYP51
3. Increased efflux of azoles from the fungal cell
4. Genomic rearrangements

The mode of azole action is inhibition of the fungal ergosterol synthesis through binding to the CYP51 enzyme leading to ergosterol depletion, cell membrane disruption, and synthesis of cell toxic sterols. Azole failure leads to ergosterol synthesis, unharmed cell membrane integrity, and reduced susceptibility to azole-induced cellular stress, and the end point is survival of the fungus.

Point mutation of the *ERG11* gene coding for CYP51 reduces the azole's affinity to CYP51. Upregulation of the *ERG11* gene increases the amount of CYP51 and enables substrate competition towards ergosterol synthesis. Upregulation of multidrug transporters reduces the intracellular azole concentration, and thereby the therapeutic dose is not achieved. Genomic alteration as chromosomal rearrangements, aneuploidy, and loss of heterozygosity causes reduced susceptibility to azole-induced cellular stress (Xie et al. 2014). All mechanisms reduce the intended effect of azoles on the fungal cell membrane and may induce multi-resistance towards azoles. The increased incidence of non-*C. albicans* infections in the Western part of the world and the increase of azole-insensitive *C. albicans* infections is believed to be a result of increased human exposure to environmental and medical azoles (Arendrup 2013).

Over-the-counter azoles for treatment of 'self-diagnosed' candidiasis, antifungal prophylaxis, and prolonged antifungal treatment contribute to the high individual exposure. However, the environmental exposure to azoles is even higher as azoles are widely used as fungicides in agriculture, industry, and domestically, e.g. seed and postharvest treatment, wood preservatives, textiles, toiletries, and human and animal excretions when in antifungal treatment (Parker et al. 2014).

The risk of iatrogenic selection of azole-insensitive *Candida* spp. in patients with recurrent oral candidiasis is reduced if the drugs for the antifungal therapy vary between different drugs over time or are combined (Xie et al. 2014). Still, caution and restriction of antifungal use are many years behind the professional and public alertness on unnecessary use of antibiotics. However, in 2013 fluconazole-resistant *Candida* was listed by the US Department of Health and Human Services as having equivalent threat level to human health as methicillin-resistant *Staphylococcus aureus* (MRSA) (Centers of Disease Control and Prevention. Antibiotic resistance threats in the United States 2013).

13.5.3 Prophylaxis of Oral Candidiasis

The majority of patients experience sporadic oral candidiasis, but some experience recurrent oral candidiasis with varying disease-free periods. Recurrent oral candidiasis might affect quality of life and oral functions because of oral symptoms, and there is a risk of infection spreading to other areas of the body. Identification of predisposing factors is the important first step followed by intervention for elimination of the predisposing factors (Table 13.3). This may not be possible in patients with local predisposing factors like oral mucosal diseases, e.g. oral lichen planus and leukoplakia, or systemic predisposing factors, e.g. disease-related or iatrogenic immunosuppression.

Depending on the type of predisposing factor, other topical treatments with antifungal effect can be successful, e.g. chlorhexidine mouth rinse in patients with oral lichen planus (see below). Prolonged or repeated treatment with antifungal drugs may be necessary, and systemic treatment with azoles is most widely used. However, there

is a risk of acquired resistance and iatrogenic selection of azole-insensitive *Candida* spp. This risk can be reduced if the drugs for the antifungal therapy vary between different drugs over time or are combined (Xie et al. 2014).

13.6 Other Topical Treatments with Antifungal Effect

In order to minimize the individual and environmental exposure to antifungals, old and new alternative agents are used or investigated for treatment and prophylaxis of oral candidiasis.

13.6.1 Chlorhexidine

Chlorhexidine is antiseptic and has a broad-spectrum antibacterial and antifungal effect. Depending on the concentration it is fungistatic or fungicidal. At high concentrations it destroys the fungus cell membrane resulting in coagulation of cellular proteins and cell death. At lower doses it inhibits the fungi adhesion to surfaces as it binds to acrylic, epithelial, and tooth structures and interacts with the fungus adhesion by modifying fungal cell surface hydrophobicity and hyphae formation. Even at low dose of long time after chlorhexidine exposure, the fungi are suppressed, which is referred to as "the post-antifungal effect of chlorhexidine" (Ellepola and Samaranayake 2001). Adverse reactions include chemical-induced epithelial desquamation, temporary brown discoloration of teeth and dentures, and taste disturbances, which are related to treatment duration and the concentration. The extent of epithelial desquamation increases with concentration at the site. Thus, chlorhexidine is not suitable for long-term treatment (Flotra 1973). In vitro it has been found that chlorhexidine and nystatin suppress each other's antifungal properties as they form chlorhexidine-nystatin complexes (Barkvoll and Attramadal 1989). Chlorhexidine is available as mouth rinse, oral gel, and cream and usually in 0.12, 0.2 and 1 % concentrations. Randomized clinical trials (RCT) have shown that chlorhexidine concentrations of 0.12 % or above successfully reduced candida yeast in bone marrow-transplanted, mechanically ventilated, and HIV patients (Lam et al. 2012). Local allergic and anaphylactic reactions are reported for chlorhexidine.

Repeated short-term oral treatment may be indicated in patients with recurrent candidiasis and poor oral hygiene, e.g. mouth rinse with 0.12 % chlorhexidine twice daily for maximum of two weeks when symptoms begin. Dentures can be soaked overnight in 0.2 % chlorhexidine after mechanical cleaning in patients with denture stomatitis (Ellepola and Samaranayake 2001).

13.6.2 Fluoride

Topical fluoride treatment affects the composition and thickness of the oral biofilm on tooth structures in particular. *Candida* is part of the oral microfilm (Williams et al. 2011). Amine fluoride and stannous fluoride have been shown in vitro to have antifungal effect close to that of chlorhexidine (Flisfisch et al. 2008). A combination of potassium fluoride and amphotericin B increases the destabilizing effect of amphotericin B on the fungal cell membrane (Li and Breaker 2012). Thus high fluoride treatment is indicated in patients with poor oral hygiene.

13.6.3 Probiotics

Probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit for the host (FAO/WHO 2001). The probiotic bacteria have been administered to patients in dairy products such as cheese, yoghurt, and milk and as pharmaceutical products as powder, lozenges, tablets, and chewing gums. In order to keep an adequate number of probiotic bacteria in the mouth, they need to be administered frequently and after oral procedures with increased oral clearance, e.g. meals and oral hygiene procedures. The *Bifidobacterium* and *Lactobacillus* are the two genera investigated in relation to oral *Candida* spp., and the oral load of *Candida* has been shown to decrease (Hatakka et al. 2007; Mendonca et al. 2012; Dos Santos et al. 2009;

Ishikawa et al. 2015; Kraft-Bodi et al. 2015). One study investigated probiotic powder for treatment of denture stomatitis, but still, the impact of probiotics on treatment and prophylaxis of oral candidiasis needs to be investigated in detail (Li et al. 2014) (see Chap. 12).

13.6.4 Essential Oils

Essential oils from plants have been investigated for antifungal alternative alone or in combination with antifungal drugs. There are challenges using essential oils. One is that genetic identical plants produce chemically different essential oils with different antifungal properties depending on growing conditions like climate, soil quality, and other external factors. Another challenge is the safety regarding allergic reaction and administration of the essential oils (Palmeira-de-Oliveira et al. 2009). In two RCT with HIV-positive patients, essential oils have been shown to reduce the candida count and oral candidiasis (Lam et al. 2012).

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