
Proceedings Paper:
Sanz, M, Beighton, D, Curtis, MA et al. (17 more authors) (2017) Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. In: Journal of Clinical Periodontology. 12th European Workshop on Periodontology, 06-09 Nov 2016, Segovia, Spain. Wiley , S5-S11.

https://doi.org/10.1111/jcpe.12682

XIII EFP Workshop jointly organized by ORCA

The boundaries between caries and periodontal diseases

Role of microbial biofilms in the maintenance of oral health and the development of dental caries and periodontal diseases. Consensus Report

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Introduction

Oral biofilm is present on all intra-oral surfaces; its composition varyies between different healthy sites and between healthy and diseased sites. Dental caries and periodontal diseases are among the most prevalent diseases of mankind (cross reference group 2). These oral diseases have a negative impact on quality of life, and dental caries and periodontitis are the main causes of tooth loss. The biofilm is an essential component involved in the development of these oral diseases. The microbial composition of the dental biofilm has been previously studied extensively mostly using cultural methods, which have a limited ability to enable the isolation of all oral microorganisms. However, recently, more sophisticated approaches have been applied, and the microbial composition of the dental biofilm has been revealed to be considerably more complex and more variable than was previously recognised. The potential interactions between individual species, between the biofilm and its environment, and between individual species and the host have been mainly studied using in vitro model systems. These models are usually unable to completely mimic all aspects of the host environment. However, in vitro studies do provide a solid knowledge base for a specific, but fragmented understanding of the potential mechanisms of microbe-microbe and microbe-host interactions that may be involved in the maintenance of oral health and the initiation and progression of dental caries and periodontal diseases [gingivitis and periodontitis].

This consensus report will provide a foundation for designing studies to elucidate the actual interactions between the biofilm and the host with the potential to design novel strategies to improve oral health.

In the context of the understanding of the role of microbial biofilms in the maintenance of oral health and the development of dental caries and periodontal diseases, the following working terms have been applied:

- Biofilm: microorganisms attached to a surface embedded in an extracellular polymeric matrix in contact with a fluid phase. The properties of the microorganisms in the biofilm are generally different from those in planktonic state.

Plaque on a tooth surface is a classical example of a biofilm, which is termed as dental biofilm. Microorganisms function in dental biofilms as interactive microbial communities. Biofilms also exist on other surfaces in the mouth and can shed into the fluid phase (saliva, gingival crevicular fluid).

- Microbial Interactions: microorganisms in biofilms are in physical proximity, which facilitates a range of interactions, which may be synergistic or antagonistic.
- Symbiosis: a mutually beneficial relationship among members of the microbial community and between the microbial communities and the host, with varying degrees of benefit.
- Dysbiosis: a change in the microbial communities associated with health, resulting in a breakdown of the beneficial relationship with the host, which is deleterious to health.
- Host defence: the host defence includes host responses and physical barriers:
  - Physical barriers, such as enamel and epithelium, together with oral fluids, protect the underlying tissues from microbial insults.
  - Host response is an active process that includes innate and adaptive immunity, which commonly arises from a microbial challenge.
- Innate host response (vs. host defence): a protective mechanism against microbial challenge that is immediate and of limited specificity.
- Adaptive host response: an acquired response to a specific microbial challenge.
- Functional fluids: fluids produced by the body that carry innate and adaptive immune mediators.

Dental Biofilm: Ecological Interactions in health and disease (E. Zaura & P. Marsh)

Multi-species biofilms form naturally on all oral surfaces. The proximity of micro-organisms to each other within these biofilms creates opportunities for species to interact. Many types of inter-microbial interactions have been described, but it should be noted that most of these interactions have been investigated in laboratory systems, and occasionally animal models, and therefore some caution should be exercised when extrapolating these findings to events in humans.

1. Are there any inter-microbial interactions that characterise a health-associated dental

biofilm?

Saliva is the primary source of nutrients for health-associated biofilms. The metabolism of host glycoproteins and proteins by the oral microbiota requires microbial co-operation and the sharing of a broad repertoire of glycosidases and proteases. The catabolism of these salivary molecules facilitates the growth of oral microorganisms but with minimal changes in environmental pH. This promotes the growth of 'neutrophilic microorganisms', i.e. species with an optimum growth in the pH range 6.5 - 7.5, resulting in microbial homeostasis in dental biofilms and the generation of a range of nutritional inter-dependencies among microbes.

Dental biofilms develop as functionally- and structurally-organised communities of interacting microorganisms. These interactions can be synergistic or antagonistic, and produce a biofilm that prevents colonization by non-oral microorganisms and provides protection to the dental surfaces.

A health-associated biofilm is associated with an active balance between slow rates of acid production and compensatory alkali generation, resulting in the biofilm environment have a neutral pH. Such conditions help to stabilise the presence of health-associated species while restricting the growth of microorganisms associated with caries and periodontal diseases.

A health-associated biofilm will produce antagonistic factors including H_2O_2, bacteriocins and other factors that may suppress the growth of microorganisms associated with disease.

2. What are the key inter-microbial interactions that define a caries-associated dental biofilm?

In addition to saliva, the major source of nutrients are dietary sugars, and the biofilm is characterised by saccharolytic, acidogenic and aciduric species, that also synthesize extracellular polysaccharides that contribute to the biofilm matrix.

The saccharolytic and acidogenic microorganisms generate a low pH from the

fermentation of dietary sugars, which inhibits ‘neutrophilic microorganisms’ associated with enamel health.

In the absence of dietary sugars, a caries-associated biofilm can generate acids from the metabolism of extracellular glucans and fructans, and intracellular storage compounds.

3. What are the key inter-microbial interactions that define a dental biofilm associated with periodontal diseases?

GCF is the main source of nutrients for microorganisms in biofilms associated with periodontal diseases, and the biofilm is characterised by proteolytic and often obligately anaerobic species.

Inter-microbial interactions are necessary for the concerted and sequential catabolism of host proteins and glycoproteins for nutritional purposes. These interactions are also essential for the acquisition of growth factors required by fastidious microorganisms present in these biofilms; examples include the release of haemin from haemoglobin, and the sharing of siderophores. Complex food webs develop, whereby the products of one organism are further degraded by neighbouring species, and species scavenge oxygen creating highly reduced environments suitable for the growth of obligately anaerobic organisms.

Some microorganisms subvert the host defences through the release of certain molecules, such as proteinases, leukotoxins, modified LPS, etc., permitting the survival and persistence of potentially susceptible species. Some of these microorganisms are not only capable of enduring the host response, but have adapted to exploit the altered environmental conditions, and are termed ‘inflammophiles’.

4. Are there inter-microbial interactions that are associated with caries and periodontal diseases?

Microorganisms associated with both caries and periodontal diseases are metabolically highly specialized and organised as multi-species microbial biofilms. Progression of both dental caries and periodontal diseases involve positive feedback loops but driven

by different stressors. In caries, the frequent exposure to dietary sugars (carbohydrates) and their fermentation to organic acids can result in positive feedback loop resulting in ever increasing proportions of acidogenic and aciduric species which increases the acidity of the environment. In gingivitis, plaque accumulation at the gingival margin leads to inflammation and a positive feedback loop resulting in ever increasing proportions of 'inflammo-philes' (Hajishengallis, 2014). Some highly specialized members of the community can subvert and dysregulate the host immune response, which may result in destruction of periodontal tissues in susceptible individuals.

Biofilms associated with caries and periodontal diseases display emergent properties, i.e. the properties of the community are more than the sum of the individual species. For example, most oral bacteria can grow only poorly or not at all in pure culture on glycoproteins in saliva or GCF, but can thrive when metabolising these complex host molecules as part of a microbial community.

Microorganisms that persist in dentin caries and periodontal diseases require the cooperative degradation of complex host molecules (proteins and glycoproteins) as nutritional and energy sources.

Concluding remarks:

Many of these interactions have been defined in vitro, using simple combination(s) of species. Additionally, the majority of the interactions studied involve bacteria only, ignoring other segments of the oral microbiota (fungi, Archaea, viruses, protozoa).

Current technological advances now enable the study of more complex community level interactions (Edlund et al., 2015), including those among members of the microbiota from different kingdoms (Diaz et al. 2016). Rather than just cataloguing which micro-organisms are present, attention needs to be focussed on what they are doing within these microbial communities (Takahashi 2015).

In future, a complete understanding of the microbial 'interactome' (Jenkinson, 2011) will allow discovery of novel ecological preventive and therapeutic approaches. Such

approaches could be applied to disease control by promoting health-associated communities through nutritional or therapeutic strategies, and interfering with the interactions associated with disease.

Role of microbial communities in the pathogenesis of periodontitis and caries (A. Mira & M. Curtis)

1. What are the microbial communities associated with periodontal health and caries free dentition?

The historical view of the microbiological community structure of healthy dental biofilms, largely based on cultural microbiology, DNA:DNA hybridization and PCR investigations, suggested that this was a relatively low diversity community in which Gram-positive organisms including streptococci and actinomyces were the predominant bacteria. More recent studies using alternative high throughput non-cultural approaches suggest the diversity in health is higher than previously thought. However, at present, it is not possible to define a "core" microbiome or specific community structures associated with health because:

- There is major variation in health-associated dental biofilm composition between different individuals (potentially reflecting different susceptibilities to disease) and between different sites in the same individual.
- There are large variations at different stages of the life course from birth through to old age reflecting changes in both oral anatomy, ecology and immune function.
- Most studies to date using the new technologies have been performed on relatively few individuals and few sites.

Nevertheless, there is an emerging consensus that several genera are normally associated with health, including Neisseria, Streptococcus, Actinomyces, Veillonella and Granulicatella. Understanding the role of these health-associated organisms may have utility in the application of pre- and probiotic approaches for the prevention and treatment of disease.

In the light of the high inter-individual variability in bacterial composition in health, it may be more helpful to focus on the functional properties of bacterial communities rather than on their taxonomic composition. Indeed, whole-DNA sequencing studies show that different healthy individuals may harbour extremely variable bacterial

communities but their gene content and functional capability are remarkably similar (Human Microbiome Project Consortium 2012).

2. What is the current understanding of the Characteristics/Functions of the dental biofilm associated with caries?

As with the position in health, there appears to be important variations in the community structure associated with caries both between individuals and at different caries sites at different stages of development in the same individual. Nevertheless there are some unifying characteristics of caries-associated biofilms. First there appears to be a reduced overall microbial diversity compared to health, which may reflect the ecological pressure of lowered environmental pH. Characteristic features of the organisms associated with caries include acidogenicity, acid tolerance and the formation of extracellular polysaccharides from dietary sucrose. In addition to the classically recognized organisms such as the mutans streptococci and lactobacilli, more recent studies implicate a wider range of organisms in a dynamic and potentially interactive community structure. These include Bifidobacterium dentium (Mantzourani et al 2009) and Scardovia wiggsiae, the latter associated with Early Childhood Caries (Tanner et al 2011). DNA from other bacteria such as Schlegelella or Pseudoramibacter appear to be present in dentin caries lesions (Simon-Soro and Mira 2015) but the lack of experimental studies on these organisms have precluded evaluation of their cariogenic potential.

3. What is the current understanding of the Characteristics/Functions of the dental biofilm associated with periodontal diseases?

The historical view of the microbiological community structure of periodontal disease, based on cultural microbiology, DNA:DNA hybridization and PCR investigations, suggested that disease is associated largely with Gram-negative proteolytic anaerobic species. Furthermore, there was a limited number of key periodontal bacterial species, which included Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia and Aggregatibacter actinomycetemcomitans, which were thought to be major pathogens. However, an emerging view using more recent technologies suggests that the microbial community structure in periodontal diseases undergoes very major

alterations – or dysbiosis - including the loss or reduction of potentially beneficial organisms, increased proportions of existing subgingival species and the appearance of a wide range of organisms which were mostly undetected by previous technologies. In the case of periodontal diseases, unlike in caries, several studies have shown that the dysbiosis is associated with an increase in microbial diversity (Dewhirst et al. 2010, Camelo-Castillo et al. 2015). This increased diversity could be the result of impaired local immune function, increased availability of nutrients or a reflection of the diverse environmental niches at the sampling site at the periodontal pocket. This view does not preclude the possibility of occasional instances of periodontitis caused by a more restricted spectrum of organisms as evidenced by the specific role of the JP2 clone of A. actinomycetemcomitans in cases of aggressive periodontitis in individuals of West African descent (Haubek et al. 2008).

The characteristics of the microbial communities in periodontal diseases will include the ability to withstand/dysregulate the immune and inflammatory response, to prevail in an anaerobic environment and to take advantage to the altered nutritional availability of potential substrates in gingival crevicular fluid flow and blood.

4. Are there common mechanism leading to dysbiosis and eventually to caries and periodontal diseases?

The microbial communities associated with periodontal health and caries free dentition are in symbiosis with the host. Maintenance of this symbiotic state will be dependent upon factors/processes derived both from bacteria (for example, nutritional interdependencies among different bacteria) and from the host (for example, salivary glycoproteins, which provide a continuous supply of nutrients to the dental biofilm). These factors/processes provide resilience to the symbiotic state and the maintenance of health. Stressors applied to the symbiotic state can perturb homeostasis and lead to dysbiosis wherein the health associated microbial population structures are significantly altered and consistent with the development of disease.

Stressors in the case of dental caries will include dietary sugars and reduced salivary flow or altered salivary composition. Stressors in the case of periodontal diseases may include alterations to the effectiveness of the immune response (for example, impaired function or reduced numbers of neutrophils) and the activities of keystone bacterial

species (for example, P. gingivalis) able to manipulate the overall bacterial population structure. The dysbiotic state may also be resilient to conversion back to symbiosis dependent upon factors derived from both bacteria and the host.

A better understanding of the nature of the factors/processes which are required for the maintenance of symbiotic and dysbiotic states and the stressors able to convert one state to another will be important for the development of preventive and therapeutic strategies in caries and periodontal diseases.

5. Which are the methods that enable the understanding of the role of the oral microbial communities?

In order to acknowledge the extensive within and between individual variations in the microbial communities in dental biofilms in both health and disease, studies with larger population sizes and more refined sampling methods are necessary. To elucidate the multi-dimensional and dynamic nature of these diseases, longitudinal investigations will be required on a range of different populations. Functional analysis of microbial populations may prove to be a more valuable approach than taxonomic analysis.

The application of '–omics' technologies coupled with methods for integration of large data sets will allow a holistic overview of these diseases which combines the contribution of not only microbial populations but also the host and the environment. Such a holistic overview may allow the translation of the multifactorial aetiology of these oral diseases into integrated diagnostic and therapeutic approaches.

With regard to understanding the functional roles of microbial populations in dental biofilms, the development and application of the following methods will be important:

- Advanced imaging at the microscopic and sub-microscopic level to elucidate, for example, community structures and cell-cell communication in both model and natural biofilms.
- Analysis of gene expression at the microbial community level in both symbiotic and dysbiotic conditions.
- Application of microbiological endpoints into randomised clinical trials.

6. How we can translate the concept of symbiosis and dysbiosis to preventive and therapeutic strategies in caries and periodontal diseases?

The historical paradigm that both caries and periodontal diseases were a consequence of only a limited repertoire of micro-organisms led to diagnostic, preventive and therapeutic strategies with only a targeted specificity. Based on our more recent understanding that both groups of diseases are associated with complex and variable communities in a dysbiotic state, future approaches should recognise this multifactorial and variable microbial aetiology and the potential functional importance of microbial populations in dysbiosis with their host.

The ultimate challenge will be to translate this new knowledge into novel, practical approaches for prevention, diagnosis and therapy of caries and periodontal diseases to the benefit of human populations.

The innate host response in caries and periodontal diseases Part 1: The role of tissues and cellular players. Functional fluids and effector molecules (M Herzberg & J Meyle)

1. What are innate host response factors associated with periodontal health and caries-free dentitions?

Antimicrobial peptides and proteins derived from epithelial cells, salivary epithelial duct cells, neutrophils, and fibroblasts provide protection for periodontal and enamel health. In addition, antimicrobial peptide rich-neutrophils are found in both saliva and gingival crevicular fluid (Dommisch and Jepsen, 2015, Gorr and Abdolhosseini, 2011). Bioactive lipids, such as resolvins, may be protective mediators in periodontal health (Hasturk and Kantarci, 2015). Mucins and agglutinins in saliva and pre-existing specific antibodies (IgG) in GCF contribute to the clearance of microbes. Other antimicrobial factors such as activated complement also function in GCF.

The dental biofilm may be affected by the relative abundance and composition of proteins in the pellicle, which could protect against caries. The salivary pellicle also contains calcium-binding proteins such as statherin, which can make the pellicle supersaturated with calcium and phosphate salts in hydroxyapatite. The pellicle can, therefore, contribute to maintaining mineralization of the enamel surface and aid in resistance to demineralization.

In response to chewing and the sensory quality of foods (gustatory stimulation), the salivary flow rate increases. The stimulated saliva has increased buffering capacity and altered composition. The increased flow tends to reduce adhesion of microbes and microbial complexes to the tooth surface. The protein composition of stimulated saliva can inhibit microbial growth, whereas the ionic content reduces enamel solubility. Collectively these mechanisms provide protection against caries.

2. What are the roles of the hard and soft tissue barriers in defence against caries? What are the roles of cells and soluble mediators of the innate response in defence against caries?

Enamel and dentin are important hard tissue barriers to the caries process. Fluoride interferes with the carious process by reducing demineralization and enhancing remineralization. Certain developmental defects of enamel result in breakdown of the barrier and may increase the risk of carious lesions. The prevalence of root caries increases in the presence of gingival recession, suggesting that intact junctional epithelium and proximal gingiva are protective.

During the caries process, odontoblasts proximal to dentinal tubules may stimulate intra-tubular mineralization resulting in closure (Garces-Ortiz et al., 2013) and reduced diffusion of intra-tubular fluid. In response, the dental pulp releases soluble pro-inflammatory mediators. For example, pulpal cells release interleukin-1β and defensins. The defensin hBD-2 and IL-1β stimulate the expression of other mediators including dentin sialophosphoprotein, contributing to dentin repair.

3. What are the roles of the hard and soft tissue barriers in defence against periodontal infections, including gingivitis and periodontitis? What are the roles of cells and soluble mediators of the innate response?

The incidence of gingivitis increases in proximity to cervical carious lesions. At these sites, the soft tissue inflammation may be promoted because dental plaque accumulates. During inflammation, the integrity of the epithelial barrier prevents bacterial penetration. Inflammation stimulates turnover of gingival and junctional epithelial cells. As the superficial epithelial cells are shed into the salivary environment and swallowed, adherent and intracellular bacteria are effectively disposed. The self-renewing quality of squamous epithelia is a valuable protective mechanism against infection.

Gingival epithelial cells also produce soluble pro-inflammatory mediators – cytokines – in response to oral microbes. For example, Gram-positive and Gram-negative bacterial pathogen-associated molecular patterns engage Toll-like receptors to signal for up-regulation of IL-1α, IL-6 and IL-8. TLR signalling and other signalling pathways work to increase innate antimicrobial resistance of the epithelium. For example, released IL-1α stimulates the epithelial cells via an autocrine loop to up-regulate the expression of antimicrobial peptides, including hBDs, calprotectin and cathelicidin.

Bacterial chemo-attractants and activated complement components stimulate activation and emigration of polymorphonuclear leukocytes (PMNs) through the junctional epithelium into the GCF. As phagocytes are storehouses of antimicrobial proteins, PMNs represent a major innate cellular response. PMNs are prominent during gingivitis and early periodontitis. A preponderance of PMNs also appears during acute exacerbations of periodontitis. Diseases affecting normal function of PMNs typically increase the risk of periodontitis. For example, periodontitis is a prominent comorbidity of Leukocyte Adhesion Deficiency Syndrome and Chronic Granulomatous Disease.

In periodontitis, the PMN response tends to reduce proportionally compared to other cells. Periodontitis is also characterized by activation of Langerhans dendritic cells

and intraepithelial lymphocytes such as γδ T cells. These innate immune cells capture and present bacterial antigens to CD4 and CD8 T cells of the adaptive immune system. Activated γδ T cells produce IL-17, which is mechanistically associated with periodontal bone loss in periodontitis.

The expression of antimicrobial peptides also differs in gingival tissues from gingivitis and periodontitis patients. In patients with periodontitis, hBD-1 levels are higher than in gingivitis. Levels of hBD-2 and hBD-3 appear similar in both infections.

4. Can we describe innate immunity associated with the caries process?

Innate immunity during the caries process includes salivary and to a lesser extent soluble mediators in GCF. The salivary factors work at the enamel surface to increase surface mineralization and minimize bacterial adhesion and growth. Within the pulp, stimulated odontoblasts also function in innate reparative mechanisms promoting mineralization from within the tooth.

5. Can we define an innate host response against dental biofilms in periodontal diseases?

The oral mucosa and transmigrating PMNs form the first line of defence against the microbial challenge arising from the dental biofilm. Antimicrobial peptides produced by epithelial cells and neutrophils defend against infection and are present in gingival crevicular fluid and saliva.

The antimicrobial role of PMNs in gingivitis tends to be replaced in periodontitis by Langerhans dendritic cells and γδ T cells. Upon stimulation, these cells increase production of pro-inflammatory cytokines including IL-1, IL-6, IL-8, IL-17, TNF-α and IL-23. The Langerhans cells and γδ T cells bridge the innate and adaptive immune responses.

Invading bacteria and their products can activate proximal capillary endothelial cells to increase expression of ICAM-1 and selectin receptors (Hajishengallis et al., 2016).

Concomitantly, capillaries allow exudation and leukocyte transmigration, facilitating the presence of leukocytes in the tissues. The activated endothelial cells also express Del-1, which blocks leukocyte adhesion to LFA-1 receptors and inhibit diapedesis. In periodontitis, Del-1 production is reduced removing an impediment to PMN infiltration of the gingiva.

Commensal bacteria and putative pathogens engage TLRs and other pathogen-related receptors (PRRs) to signal for up-regulation of key cytokines and antimicrobial peptides. For example, bacterial lipopolysaccharide (LPS) is bound to LPS-binding protein (CD14) and selects TLRs on neutrophils, monocytes, macrophages and mast cells. Engagement of LPS activates these leukocytes, which reside in the subgingival connective tissue. The antimicrobial peptide LL-37 may antagonize the action of LPS.

Antimicrobial peptides can also serve as danger-associated molecular patterns (DAMPs), which activate inflammasomes of the epithelial cells and PMNs. A major product of the inflammasome, superoxides, is toxic to both microbes and host tissues. While antagonizing the growth of bacteria, superoxides may cause local tissue damage and micro-abscess formation.

Prostaglandin E2 and related lipid mediators can activate immune cells and stimulate pro-inflammatory and pro-coagulant responses. In contrast, tissue destruction during inflammation in periodontitis appears to be somewhat reversible. Tissue damage appears to be reversed by resolving lipids such as the resolvins and maresins.

Complement proteins in GCF percolate through the junctional epithelium into the gingival sulcus. Rapidly activated by oral microbes, complement peptides elicit an inflammatory response. For example, C3b acts as an opsonin, facilitating phagocytosis and intracellular killing of microbes by neutrophils.

7. Describe the innate host response when caries and periodontal diseases co-exist?

Given that the present state of knowledge suggests that the aetiologies of caries and periodontal diseases are mutually independent, the elements of innate immunity that appear to contribute to resistance to both are somewhat coincidental.

8. Which mediators in dental biofilms, saliva or gingival crevicular fluid suggest greater susceptibility to caries, periodontal diseases or both?

Longitudinal prospective studies are required to obtain data to identify cellular and/or soluble mediators of innate immunity that might serve as biomarkers of disease.

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