

Microbiology of Dental Caries: A Literature Review

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Abstract

Aim: to review the current knowledge on dental caries microbiology and critically appraise the literature. **Methodology:** An electronic search of the available dental literature was done using different databases (PubMed, Google Scholar, Scopus). The following keywords were used: dental caries, carcinogenic bacteria and oral biofilm. In addition, classic textbooks that related to dental caries and oral microbiology were searched. **Findings:** Oral bacteria can grow in two ways: in planktonic or biofilm forms. In the past, most microbial studies were studies of the planktonic form. Only around 0.1% of oral bacteria grow in the planktonic state. Bacterial cells usually aggregate and attach to the tooth surface to form an oral biofilm. The polysaccharides in the biofilm matrix are a resource that acidogenic bacteria metabolise and produce acids to initiate or progress the dental caries lesion. Different types of acids are created as a result of biofilm metabolism, which cause a shift in oral pH under the critical level. This reduction in the pH level will influence the chemical composition of the tooth surface. The bacterial cells start to multiply to form a microcolony within 24 hours. If left undisturbed the growth is continued, resulting in a mature type of biofilm within a week. **Summary of findings:** The most frequent species found associated with dental caries were mutans streptococci and *lactobacilli*, which shift the balance towards tooth tissue demineralisation.

Keywords: Dental caries; Carcinogenic bacteria; Oral biofilm

Introduction

This paper focuses on the microbiology of dental caries. It is important to give a brief introduction to dental caries for the reason of compliance. Dental caries can be defined as a localised and chemical loss of the tooth structure caused by the metabolic activity of dental biofilm that covers the tooth surface.^[1] This loss is reversible, especially in its early stages. The final outcome of dental caries is determined by multiple factors such as saliva, exposure to fluoride, and sugar dietary consumptions, which influence the dynamic balance between the demineralisation and remineralisation processes.^[2] Dental caries can be developed in any part of the tooth surface: enamel, dentine or cementum. However, it is more common in the sites wherein the dental biofilm is more protected, which allows it to mature and grow. Examples of these sites include: grooves, fissures, and pits on the occlusal surface and along the gingival margin.

Literature Review

The mouth, as with other parts of the human body, is a habitat for resident bacteria, which are called microbiota.^[3] They are not living passively, but rather contribute to maintaining the body's health by contributing to immune system development and excluding pathogenic, exogenic microorganisms.^[4] This is called colonisation resistance and it happens as the resident microbiota are more competitive regarding attachment to the oral receptors and nutrient acquisition. The application of the molecular approach to identifying the resident oral microbiota brought an opportunity to estimate the high number of

microbiota in the oral cavity. It is estimated that around 700 different species live in the mouth.^[5]

The attempts to understand dental caries aetiology started in 5000 BC, when the cause of dental caries was described as a tooth worm. This theory of the tooth worm was rejected by Pierre Fauchard, who is known as the father of modern dentistry. In the 1680s, van Leeuwenhoek discovered microorganisms in the tartar taken from his teeth. He drew the microbes in his notebook, which are known now as cocci, fusiform and spirochetes bacteria.^[6]

The development of caries was believed to be caused by only a few gram-positive bacterial species, such as *Streptococcus mutans*, *Streptococcus sobrinus* and *lactobacillus*.^[7] This understanding was based on cultivation studies by isolating these bacteria and determining their cariogenic properties. This is called the specific plaque hypothesis. It became evident that a caries lesion could happen in the absence of these putative pathogens. Current evidence states that population groups and individuals are susceptible to dental caries with a low level of *S. mutans*, and vice versa.

This led to the formulation of another hypothesis that attempts to

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explore dental caries aetiology: the ecological plaque hypothesis by Phil Marsh. This hypothesis proposed that the shift in the resident bacterial species' balance, which responds to changes in local environmental conditions, dysbiosis, is responsible for the disease. In addition, the microbiota associated with diseases may also be found in healthy sites at levels too low to be critical.^[8] The interaction between the biofilm bacterial species that determine the biofilm's properties, e.g. repeated low pH conditions following sugar intake, favours the growth of acid-tolerant and acid-producing bacterial species, which leads to a shift in the bacterial community, being dominated by acidogenic gram-positive bacteria (such as *Lactobacilli* and *S. mutans*) at the tooth surface.^[8,9]

A metagenomic study that aimed to investigate the microbe in the dental caries cavity revealed that the caries cavity is dominated by a complex community of many bacterial species rather than *S. mutans*.^[10] In addition, a study of the oral microbiota of dental caries in children showed that the plaque with *Veillonella*, *Streptococcus*, *Leptotrichia*, *Actinomyces*, *Granulicatella* and *Thiomonas* was associated with dental caries significantly.^[11] Another investigation aimed to investigate the salivary microbiome of caries-active participants; it showed that the occurrence of dental caries was supported by shifts in the bacterial community structure rather than the absence or presence of a specific bacterial species.^[12] These data support that no single specific pathogen has a correlation with dental caries but rather the polymicrobial aetiology of caries. Thus, in dental caries it is now recognised that it is not caused solely by the presence of a single bacterial species, such as *S. mutans*; rather, it is the result of the interaction between multiple acidogenic bacteria within the dental plaque.

Bacteria can grow in two different ways: in a planktonic or biofilm form. In the past, most of the microbiology studies were conducted in liquid culture wherein the bacteria were free to float. Even though during that period a fundamental of microbiology work was accomplished, it is estimated that the percentage of bacteria that grow in a planktonic form is only less than 0.1%. Therefore, how do these bacteria grow in the oral cavity? They grow in a very sophisticated microbial structure called biofilm.^[8]

It has been established that oral microbial biofilms establish themselves on oral surfaces, namely tooth surfaces. The oral biofilm exists in a balanced equilibrium with host defences and is important in maintaining oral tissue integrity. The disease occurs when this balance is disturbed to the benefit of the biofilm. In fact, 65-80% of bacterial infections affecting humans are caused by biofilm infections.^[13] This gives importance to studying the biofilm properties in all medical and dental microbiology sectors.

The oral biofilm defined as aggregates of bacteria cells which adhere to a surface, such as a tooth surface; these cells are embedded in a matrix of extracellular polymeric substances which helps to be more tolerant towards host and antimicrobial defences.

When the teeth are exposed to the oral environment, within a few seconds the tooth surfaces are covered with a film called the acquired pellicle, which contains proteins and glycoproteins that derive from saliva and gingival crevicular fluid.^[14] After the acquired pellicle is formed, a few bacterial species start attaching to the pellicle. The initial colonisers, mostly *Streptococcus mitis* and *S. oralis*, form a loose, irreversible bond to the acquired pellicle. As the oral biofilm continues the process of proliferation, the metabolism of pioneer bacterial species modifies the local environmental conditions to favour the secondary colonisers that adhere to the early colonisers through a process called coaggregation or coadhesion. This process is known as coaggregation, which means the adhesion of bacteria.^[15] The oral biofilm is a dynamic and complex structure. In some cases, more than 50 species could be found in one site.^[16]

In addition to bacterial cells, an important component of biofilm, which is considered the structural backbone, is the extracellular matrix. It is composed of an extracellular polymer substance known as Extracellular polymeric substances EPS. It is not only important as a physical scaffold with which to determine the structure of biofilm, but also biologically active and able to maintain water, nutrients and important enzymes.

Microbiota of dental caries

Dental caries happens mainly when the production of organic acid results in dental hard tissue decalcification. Thus, dental caries bacteria should be acid-tolerant in carrying out the dental caries process in an acidic environment.^[17] Acidogenic bacteria, e.g. *S. mutans*, are able to function at pH 6 and can carry out the glycolysis process at pH 4 or below. *Lactobacillus* spp., considered a moderate acidophilic bacterium, can function at a pH level from 3-4.^[3] In general, the acid tolerance property allows cariogenic bacteria to displace other acid-sensitive bacterial species, which would lead to enriching the aciduric bacteria and continuing the acidification process of dental caries, which is favourable for caries formation.^[18]

Dental caries development occurs when acidic microbial metabolites form carbohydrate substrates.^[19] Lactic acid has been identified as the major contributor to declining pH in dental plaque.^[17] The accumulation of acids leads to a long pH decline of the critical pH, which contributes to tooth hard tissue demineralisation.^[8] Therefore, this paper will be focused on the major acidogenic bacteria in this section, these are: *Streptococcus mutans*, *Lactobacilli* spp. and *Actinomyces* spp.

It was first discovered by J. Kilian Clarke in 1924.^[20] *S. mutans* is a gram-positive facultative coccus that is usually arranged in chains. Oral streptococci are considered commensal bacteria, but they are opportunistic as well, which makes them able to initiate dental caries.^[21] Mutans streptococci are a group of bacteria that are associated with dental caries. They consist of: *S. mutans*, *Streptococcus sobrinus*, *Streptococcus rattus*, *Streptococcus cricetus*, *Streptococcus ferus*, *Streptococcus downei*, and *Streptococcus macaca*. *S. mutans* cariogenic virulence involves many attributes [Table 1] adhesion for initial attachment to the saliva-coated tooth surface, production

of extracellular polysaccharides such as glucans and fructans to increase retention on the tooth surface and facilitate plaque accumulation, and production of organic acid to produce an acidic environment and support aciduric microflora.^[22]

A study was conducted to determine whether the acidogenicity and acid tolerance of *S. oralis* and *S. mitis* correlate with early stages of enamel caries or healthy individuals.^[21] The authors concluded that the positive correlation between acid tolerance and acidogenicity characteristics of *S. mitis* and *S. oralis* with dental caries was not recognised in this study [Table 2]. The samples of this study were collected from the occlusal surfaces of second molars, which have been banked as part of another study. The samples do not properly represent the biofilm in the oral cavity as they grew in a laboratory environment and were collected from a specific area on the teeth, which might be considered biased. In addition, the time of the incubation of these samples has not been mentioned in the study, which might affect the growth of bacterial species even if they try to mimic the oral environment. This study used Polymerase chain reaction (PCR) to identify bacteria, this method is a highly sensitive technique and any contamination of the sample even with a small amount of DNA could mislead the results. Another limitation is that PCR can only identify the absence or presence of a specific pathogen or gene which might be biased the result.^[23]

A recent study aimed to address the individual effects of each species in the oral biofilm in causing dental caries.^[22] The study included five bacterial species in addition to *S. mutans*; they were divided into six different groups: a control group contained the six different bacterial species and the other groups have one of the bacteria species absent. The biofilms in this study were

grown in hydroxyapatite disks that had been preconditioned for pellicle formation in a pool of saliva so as to initiate the biofilm. Growing biofilms in a laboratory environment excluded other factors, such as carbohydrate consumption and the difference between individuals in saliva production and constituents, which could have affected biofilm formation in the oral environment. The authors concluded that the absence of *S. oralis* and *A. oralis*, which are considered commensal bacterial species, leads to *S. mutans* overgrowth in the biofilm [Table 2]. This study result was in agreement with another study that found that *S. mutans* had a significant high proportion in the biofilm collected from caries-active dental sites.^[24] The greater number of *Streptococcus mutans* in the dental caries sites result in their ability to produce acids at faster rates than those of other microbiota.

Lactobacilli spp. are gram-positive facultative anaerobic rod-shaped bacteria which are able to convert lactose and other sugar substrates to lactic acid and survive in a low-pH environment (as low as 3).^[25] It is a poor coloniser on a smooth tooth surface; thus, it is believed that *lactobacilli* exacerbate the initial dental caries in the enamel surface to a deep dentine caries lesion, and they are considered secondary invaders rather than initiators of caries.^[26] Many studies have shown a relationship between dental caries and the presence of *lactobacilli* in children younger than 6 years with active dental caries.^[27,28] *S. mutans* and other acidogenic oral bacteria are mechanically able to retain *lactobacilli* and provide a physical containment area with a low-pH and anaerobic environment, which is preferable to anaerobic bacteria.^[25]

Table 1: Virulence factors of *S. mutans* that contribute to their cariogenicity.

Property	Description
Acid production	Ability to produce organic acids, mainly lactic acid
Sugar transportation	Ability to metabolise sucrose to form insoluble polysaccharides, which helps in colonisation persistence on tooth surfaces.
Aciduricity	Ability to tolerate environmental stresses such as low pH, which is considered a toxic environment for other bacterial species in the mouth
Intracellular polysaccharide production (IPS)	Ability to use IPS to continue producing acids in the absence of dietary sugars
Extracellular polysaccharide production (EPS)	Contributes to the biofilm matrix, localises acidic fermentation products and consolidates cell attachment

Table 2: Summary of study methods and findings.

Author(s)/Year of publication	Method of identification	Sample size	Findings
Banas et al. (2016)	PCR ¹	85 subjects (38 males, 47 females)	Positive correlation between acid tolerance and acidogenicity prosperities of <i>S. mitis</i> and <i>S. oralis</i> and caries was not observed
Thurnheer & Belibasakis (2018)	FISH ² CLSM ³	7 different inocula with different strains	Absence of <i>S. oralis</i> and <i>A. oralis</i> leads to <i>S. mutans</i> overgrowth in the biofilm
Yang et al. (2010)	16S RNA using <i>Lactobacillus</i> -specific primers	7 children (42 samples)	Absence of <i>Lactobacillus</i> in active dental caries cavities
Piwat et al. (2010)	16S RNA, PCR	59 children	<i>Lactobacillus</i> exhibited wide species and genotype heterogeneity. <i>Lactobacillus salivarius</i> were dominant in children with a high level of caries
Dame-Teixeira et al. (2016)	RNA-sequencing approach	Sound root surface=10, Active root caries=9	Similar levels of <i>Actinomyces</i> gene in both carious root biofilms and sound root surfaces
Benitez-Paez et al. (2014)	RNA-sequencing approach	28 teeth	Bacterial activity changes during biofilm formation and after food ingestion were individual-specific

¹Polymerase chain reaction, ²Staining of biofilms by fluorescence *in situ* hybridisation, ³Visualisation of biofilm by confocal laser scanning microscopy

On the contrary, another study failed to detect *lactobacilli* in children with active dental caries.^[12] This study used *Lactobacillus*-specific primers to conduct the 16S RNA test, which could be more informative in detecting *Lactobacillus* than bacterial universal primers, which are used in studies detecting the presence of *Lactobacillus* with active dental caries [Table 2]. However, this study included seven children, which is considered a very small sample size; in addition, they stated that the sample was collected from the posterior teeth by using only a sterilised diamond bur with no clear description of the method. Failing to prevent any contamination of the diamond bur could affect the results.

These previous studies were conducted with a cross-sectional design, with the samples being collected at a single time point. Besides having advantages of analysing a large number of sites and people, different patient ages, ethnicities, genders and different tooth surfaces can be analysed; detecting *Lactobacillus* in the oral cavity was not given a definitive conclusion as to whether it was able to cause or progress a dental caries lesion or it was transient contamination from other sources with no main role in dental caries. Only associations between microbiota and dental caries, not causation, can be derived from these studies. Even the count of *Lactobacilli* increased in sites of the caries lesion; their proportion to other microbiota species in the biofilm is unknown.

Actinomyces spp. are gram-positive facultative or strict anaerobic rod-shaped bacteria.^[29] There is a suggestion that there is an association between *Actinomyces* spp. and dental root caries based on studies that isolate *Actinomyces* spp. from teeth with a root caries lesion. Many studies have investigated the association between bacteria and dental caries in a root surface using a culture-dependent approach aiming to detect the root caries pathogen.^[24,30] A culture-dependent approach could detect the presence or absence of the microorganism; however, it is not possible to determine whether the bacterial cells are viable or not and whether it is not feasible to determine the bacteria cells' virulence factors and their contribution to caries development.^[10]

A recent study aimed to determine the transcriptional dynamic of *Actinomyces* spp. in both health and disease root surfaces. The study concluded that the metabolic activity of *Actinomyces* was very similar in both caries and health root surface samples. Even though this study used an RNA-sequencing approach, which is able to study gene expression which leads to the assessment of bacterial cell functions, the sample size in this study is too small as it included only nine root caries lesions. In addition, a detailed description of the sample collection is lacking in their transcript, which makes the reproducibility of this study difficult. The authors stated that the disease root surface samples were collected from active caries lesions; it is difficult to determine whether the lesions at the time of sampling were progressing or arresting or whether the lesions had different species in different stages because they were collected at one time only. One important limitation of RNA-sequencing approach is its inability to provide a quantitative view of the pathogen so the number reads obtained by this method does not

necessarily correlate with the actual pathogen's abundance.^[31] Table 2 shows a summary of six studies' findings that conducted to detect dental caries microbiota via different methods.

The new direction of ongoing oral dental plaque studies is focused on oral microbiome and metagenomic approaches. Metagenomics is defined as 'the application of modern genomics techniques to the study of communities of microbial organisms directly in their natural environments, bypassing the need for isolation and lab cultivation of individual species'.^[32] The metabolic function analyses conducted on genes via metagenomic techniques help researchers to identify the metabolic processes and functions of particular bacterial communities.^[33]

Discussion and Conclusion

Although there are studies showing an association between dental caries and *S. mutans*, *lactobacilli* and *Actinomyces*, most of the study samples were collected from caries sites wherein these groups of bacteria cannot be isolated. This could suggest that other microorganisms can contribute to the dental caries process. Therefore, it is the change in dental plaque ecology that leads to caries lesion development. Whenever there are non-shedding surfaces, bacterial cells will attach to saliva film called the pellicle; the bacterial cells start to multiply to form a microcolony. If left undisturbed the growth continues, resulting in a mature type of biofilm within a week. Different types of acids are created as a result of biofilm metabolism, which causes a shift in oral pH under the critical level. This reduction in the pH level will influence the chemical composition of the tooth surface. The cumulative result of many pH fluctuations over a long period of time is the loss of calcium and phosphate, which makes the enamel surface seem porous (which can be detected clinically as a white spot lesion). Thus, the caries lesion is a result of an imbalance in the equilibrium between tooth mineral loss and biofilm fluid.

Competing Interest

The authors declare that they have no competing interests.

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