Early childhood caries - from materia alba to the oral microbiome

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INTRODUCTION

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]. When appearing in infants and toddlers, early childhood caries (ECC) is a complex condition associated with impaired oral health-related quality of life and high costs for families, as well as society. There are numerous biological, medical, behavioral, psychological, cultural, and life-style factors associated with the disease that together with deficient parental support are behind its etiology. Fluoridated toothpaste and fluoride varnish together with motivational interviewing are currently the most effective strategies to prevent ECC while the treatment relies on sealants, temporary restorations, and traditional restorative care [Twetman and Dhar, 2015]. This paper will focus on the microbial aspects of the disease and recent insights in the oral microbiome in relation to early childhood caries.

WHAT IS A BIOFILM?

It is nowadays well established and generally recognized that bacteria play an important role in health and wellbeing. Many terms have been used to describe the accumulation of debris in the oral cavity. For many years, it was named *materia alba* ("the white materia") although it was known to consist of bacteria accumulated microorganisms, desquamated epithelial cells, blood cells, and food debris. This term was later replaced by the expression *dental plaque* being a dense, non-mineralized, highly organized *biofilm* of microbes, organic and inorganic material derived from the saliva, gingival crevicular fluid, and bacterial byproducts. Plaque is probably still the most commonly used expression among dental professionals and their patients. It is also well established in the context of the non-specific and specific plaque hypothesis that earlier were used to explain the pathogenesis of dental caries. Since dental plaque is one of the human biofilms, this term is now more commonly applied among researchers based on the increasing understanding of its complex properties. A biofilm is any aggregate of microorganisms in which cells adhere to each other on a surface that is protected. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance. Biofilms are present over the entire body and are formed in a dynamic equilibrium with the environment. An interesting detail for dentists is that the dental plaque is one of the easiest accessible biofilms in the body and thereby increasingly attractive and of interest for interdisciplinary researchers. The properties of biofilm communities are more than the sum the component microorganisms [Marsh et al., 2015]. For example, a biofilm can be more than 100 times more resistant to antibacterial agents than planktonic cells. In general, biofilms that are diverse and stable are associated with health (homeostasis) while destabilized biofilms with a reduced diversity (dysbiosis) are associated with disease. The resident oral microbiota is normally diverse and beneficial to the host but the stability can be disrupted by stress. With respect to caries, the repeated exposure to dietary sugars, and hence

Table 1. Factors that may affect the composition of the oral biofilm

Variable	Examples
Genetics	bacterial exchange of genetic material through plasmids
Location, oral cavity	mucosa, tongue, sub-gingival pocket, access to saliva
Location, teeth	occlusal fissure, interdental area, smooth surface
Age	biofilms alter with age, degeneration of glands
Diet	sugar rich diet vs. beans, fruit and vegetables
Smoking	non-smokers vs. smokers
Alcohol	excessive intake may affect composition
Medication	xerogenic drugs, drug abuse
Sexual behavior	kisses, and more

subsequent 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]. This ecological shift of the commensal microbiota is called the ecological plaque hypothesis. Thus, caries is not a classical infection but should be regarded and handled as a non-communicable disease. It is however important to stress that a stress-derived dysbiosis is reversible; when appropriate actions are taken to counteract the environmental challenge, biofilm may return to stability through a "colonization memory" in the biofilm. From a pure chemical point of view, caries is an imbalance between mineral loss and mineral gain. When more minerals are lost over time, a caries lesion eventually becomes visible. In a simplified way, demineralisation occurs at low pH conditions in the oral biofilm (plaque) and remineralisation at pH levels around neutral and above. If left untreated, the initial lesion will progress in stages and progress to moderate and advanced lesions. The progression rate is generally faster in the primary dentition as compared with permanent teeth. Severe early childhood caries displays the most rapid and rampant progression of the caries disease with advanced cavities before the age of 3 years.

COMPOSITION OF THE ORAL BIOFILM

The oral cavity contains hundreds of bacterial species that together with fungal and viral inhabitants form highly complex communities where they interact with each other and with the host. Each person hosts around 1.5-2 kg of bacteria and there are more bacteria in 1 mg plaque (10^{12} cfu) than people on earth. This equalizes the amount in the intestines but the proportion of the taxa Firmicutes is higher in the oral cavity. One ml of saliva contains around 10^{8} colony forming units of bacteria. The Human Oral Microbiome Database contains over 600 taxa and 13 phyla [Dewhirst et al., 2010]. A "healthy" core oral microbiome consisting of 12 genera that is shared by most humans has been proposed by Zaura and co-workers [2009]; *Streptococcus, Veillonella, Granulicatella, Neisseria, Haemophilus, Corynebacterium, Rothia, Actinomyces, Prevotella, Capnocythophaga, Porphyromonas*, and *Fusobacterium*. The supra-gingival biofilm is dominated by Gram positive species while Gram negative strains are more prevalent in sub-gingival biofilms. It is however important to understand that the oral biofilm is individual and unique for each person and the composition is depending on a number of factors as listed in Table 1. In this context, the first years of life seem to be of special interest as the fetal tolerance toward the mother's microbiota during pregnancy is a major factor selecting for the acquisition of the oral microbiome.

BIOFILMS IN EARLY AGE

The oral cavity is colonized by bacteria immediately upon birth. However, the sequence and timing of microbes dictate the composition of the oral biofilm on a "first come, first served" basis. For example, children born through vaginal delivery have more beneficial bacteria and a greater diversity in their oral biofilms than children delivered with caesarian section [Nelun Barfod et al., 2011; Lif Holgerson et al., 2011]. The richness of species and taxa diversity increases with age [Lif Holgerson et al., 2015] but differences due to the mode of delivery may persist up to at least one

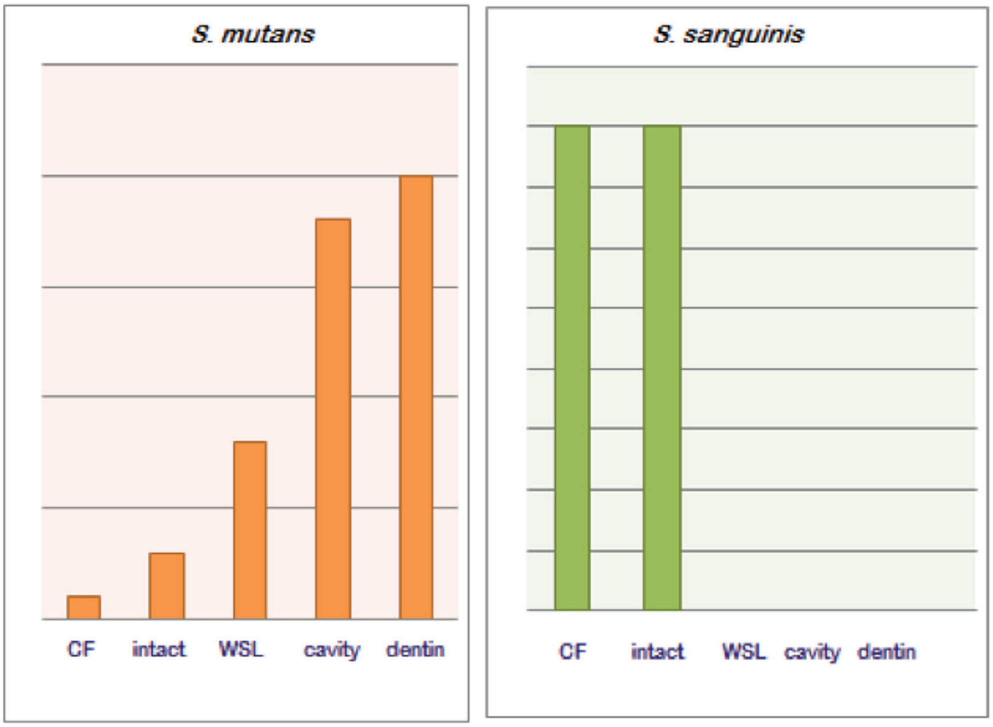

Figure 1. Presence of S. mutans and S. sanguinis in early childhood caries. S. mutans are rarely detected in caries-free subjects (CF) or from intact tooth surfaces (intact). A dramatic increase is however displayed at sites with early enamel lesions (white spots, WSL), cavitated lesions and dentin caries. On the other hand, S. sanguinis are only recovered in caries free subjects (CF) and from clinically intact surfaces. In sites with progressing disease, the bacteria are harbored in non-detectable levels. Data derived from Becker at al., 2002.

year of age. Consequently, it has been reported that children born via caesarian section display more caries at the age of three years than vaginally delivered children [Barfod et al., 2012]. Interestingly, there is a general link between the mothers and their infants at 6 month of age concerning the biofilm composition but the similarities seem to be greater among vaginally delivered children (Boustedt et al., 2015]. Another example is that breast-fed infants are reported to have a higher diversity than formula-fed at three months of age [Holgerson et al., 2013] and this is likely explained by the high content of oligosaccharides that act as prebiotics by promoting the growth of beneficial bacteria. According to the hygiene hypothesis, there is a danger of an over-clean environment; children should be exposed to as many beneficial bacteria as possible early in life in order to stimulate the development of the immune system and prevent chronic conditions such as allergies and asthma. An interesting observation concerning the use of pacifiers has been reported by Hesselmar et al. [2013]. Children whose parents "cleaned" their children's pacifiers by sucking had a greater microbial diversity in their saliva than children whose parents did not. Moreover, this parental sucking resulted in a significantly reduced risk for allergy and eczema. A vision is that transfer of oral microbes from parent to infant via the pacifier can be used in primary prevention of general and dental diseases in the future.

BIOFILMS AND EARLY CHILDHOOD CARIES

The microbiology in relation to early childhood caries has been extensively studied, initially with conventional agar-based methods. Mutans streptococci can colonize the oral cavity of predentate children and it seems clear that an early acquisition of S. mutans is associated with an increased risk of early childhood caries and further caries development later in life [Thenish et al., 2006; Parisotto et al., 2010]. The bacteria are most often derived from the mothers through a vertical transmission [da Silva Bastos Vde et al., 2015]. The risk of transmission increases with high maternal salivary levels of mutans streptococci and frequent inoculations through kissing and sharing spoons, etc. Furthermore, an explanatory study has suggested that the presence of mutans streptococci could be associated with the race of the child, number of teeth present, presence of cavitated lesions, proportion of teeth with plaque, and lower levels of maternal education [Weber-Gasparoni et al., 2012]. In fact, maternal salivary bacterial challenge was not only is associated with oral carriage among children but could also predict early childhood caries occurrence in a high-risk cohort of 36-month-old children [Chaffee et al., 2014]. It is should however be emphasized that horizontal transmissions of bacteria also may occur [Berkowitz, 2006].

In recent years, genetic technologies have been increasingly used to analyze the oral microbiota. The two most common methods are sequencing of the 16S rRNA gene and deep metagenomics sequencing. The former approach is used to answer questions on "Who is there?" while the metagenomics can address biofilm functions ("What are they doing?"). The findings of the molecular-based studies have confirmed the importance of *S. mutans* for the development of early childhood caries and the expanded the insights into the ECC-associated biofilms. Although the microbiota is highly complex, the major species associated with early childhood caries included *Streptococcus mutans, Scardovia wiggsiae, Veillonella parvula, Streptococcus cristatus,* and *Actinomyces gerensceriae* [Tanner et al., 2011]. Among them, *S. wiggsiae* is recognized as a "new" caries pathogen significantly associated with childhood caries both in the presence and absence of *S. mutans* [Tanner et al., 2011]. Furthermore, it has been shown that several genera including *Streptococcus, Granulicatella, Actinomyces* and *Porphyromonas* are strongly associated with severe early childhood caries [Jiang et al., 2013]. Therefore, it has been suggested that increased levels of those genera can be potential biomarkers of dental caries in the primary dentition [Ma et al., 2015]. In children with early caries, the diversity of the bacterial communities was reduced in comparison with to caries-free children and many species occurred at lower levels or non-detectable levels as caries advanced, including the *Streptococcus mitis* group, *Neisseria,* and *Streptococcus sanguinis* [Gross et al., 2012]. An example of such an over- and under-abundance of *S. mutans* and *S. sanguinis* in relation to early childhood caries is illustrated in Figure 1.

ANTIBACTERIAL METHODS TO PREVENT AND TREAT ECC

Several antibacterial agents have been evaluated in order to prevent and combat early childhood caries development, including topical applications of chlorhexidine, povidone iodine, xylitol and silver diamine [Twetman and Dhar, 2015]. However, based recent reviews of literature, such antimicrobial interventions and treatments showed only temporary reductions in mutans streptococci colonization levels and the effect on cavity formation was found to be minimal, if any [Li and Tanner, 2015; Twetman and Dhar, 2015]. Thus, there is insufficient evidence to support the use of antibacterial agents for caries prevention in young children. However, a proactive approach is to interfere with the maternal transmission of mutans streptococci and delay the acquisition of aciduric and caries-associated bacteria. A recent meta-analysis has displayed that habitual xylitol consumption from chewing gums or tablets by mothers with high mutans streptococci levels was associated with a significant reduction in the mother-child transmission of salivary mutans streptococci [Lin et al., 2015]. However, the concept requires a screening procedure of prospecting mothers as well as a high compliance for long periods. Furthermore, the long-term effects and cost effectiveness have been questioned and remain unclear [Thorild et al., 2013; Laitala et al., 2013]. An alternative and emerging concept to prevent early childhood caries is to expose new born infants and toddlers to beneficial bacteria. The definition of beneficial or probiotic ("for life") bacteria according to WHO/FAO is "live microorganisms which when administered in adequate amounts confer a health benefit on the host". In a recent study, caries development in the primary dentition was compared between children that got five daily drops of the probiotic bacterium *L. reuteri* during their first 12 months of life, or placebo [Stensson et al., 2014]. The results displayed significantly less caries at 9 years of age. Although a similar project with *L. paracasei* was less successful [Hasslöf et al., 2013], the findings illustrate the potential of modification of the biofilm at an early age. Probiotic bacteria in lozenges have also been proven to significantly reduce caries increment in 2 year-old children living in a low socioeconomic immigrant area with a high caries risk [Hedayati-Hajikand et al., 2015]. Although it is still too early for certain clinical recommendations, the strategy of using probiotic bacteria early in life is promising and merits further well-performed clinical trials to further elucidate its clinical applications.

The current metagenomics sequencing can today be used to create a gene catalogue of the oral microbiota and allow functional analyses of the microbial community in health and disease. Eventually, this will be helpful in elucidating the microbial pathogenesis of early childhood caries and hopefully, open up a novel landscape for preventive and therapeutic approaches for the management of early childhood caries in the near future. The advances in biofilm engineering may also assist in reduction of antibiotic resistance and aid the development of new and gentle antimicrobials.

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