Application of modern research techniques, has allowed fuller understanding of many important phe- nomena occurring on the tooth surface near the gingi- val margin and in the gingival sulcus, the site of bacte- rial dental calculus deposits.This techniques has also contributed to the knowledge of the highly complex structure of dental plaque.Supragingival plaque is dominated by Gram positive bacteria, including Streptococcus mutans, Streptococcus salivarius Streptococcus mitis and Lactobacillus, while subgingival plaque is dominated by Gram-negative anaerobic bacteria, such as Actinobacillus, Campylo- bacter spp., Fusubacterium nucleatum, Porphyromonas gingivalis (Hojo et al., 2009; He and Shi, 2009; Marsh, 2012).Several salivary components have been shown to have a role in microbial adhesion to the pellicle, for instance, salivary oligosaccharide-containing glyco- proteins may serve as receptors for oral streptococci and the salivary proline-rich protein 1 and statherin have been implicated as receptors for type 1 fimbrie of Actinomyces viscosus (Gibbons et al., 1988; Marsh, 2005; Scheie and Petersen, 2004).In the initial non-specific phase of biofilm forma- tion, when bacteria that form it are located at a consid- erable distance apart, the electrostatic, hydrophobic and van der Waals forces allow for reversible adhesion of microorganisms (Hics et al., 2003; Scheie and Petersen, 2004).The formation and development of biofilm takes place in three main steps: attachment of the initial pio- neer species, which leads to an increase in the biofilm mass due to colonization, co-adhesion, co-aggregation of other species of microorganisms, production of extracellular polysaccharides and separation of bacte- ria from the surface of the biofilm and their spread in the environment of the oral cavity.Gingival fluid is composed of different host-derived molecules rich in proline, tyrosine, histidine, includ- ing proteins and agglutinins which act as a source of receptors that are recognized by various oral bacteria, mucins and other glycoproteins.A major role in the formation of biofilm is attrib- uted to extracellular polysaccharides (EPS: Exopolysac- charides) containing, among others, mannose and gly- cosidic residues, which form a bacterial capsule or are released into the environment, where they become part of mucus.A particu- larly important role in the creation of plaque mass and adhesion of bacteria is attributed to polysaccharide polymers-soluble ones such as glucan and fructan, and insoluble ones, such as mutan (Hojo et al., 2009; Marsh, 2011).As the attached bacteria cells grow and divide, many will start to express a biofilm phenotype, which will include the secretion of extracellular polymeric sub- stances (EPS) with polypeptides, carbohydrates and nucleic acids (Hojo, 2009; Kolebrander et al., 2010).The wide range of streptococcal species produce the most well-studied oral adhesins, including antigen I/II, PaG, SspA, amyl- ase-binding proteins, and type 1 fimbrie-associated protein.Dental plaque was one of

2 The oral microbiome in dental caries 131 the first structures formed by bacteria that has been described as a biofilm (Marsh, 2005; ten Cate, 2006; Hoiby et al., 2011).Many years of research have shown that this biofilm is a highly specialized, co-ordinated, multi-species form of microorganism life, permanently located on the tooth surface in a matrix, surrounded by a layer of extracel- lular polysaccharides (EPS).Microorganisms in the oral cavity form two types of biofilm on the surface of the tooth: the supra-gin- gival plaque and the sub-gingival plaque, which differ significantly in the composition of the bacterial flora.The initial stage of bac- terial adhesion to the tooth surface includes interaction of the superficial substances of microorganism with the components of saliva contained in the acquired pellicle.For example, due to changes in the environment such as limited access to oxygen, the

metabolism of cells changes: the activity of anaerobic metabolic pathways of glycolysis increases, and the synthesis of certain enzymes is inhibited (Ling et al., 2010; Roberts and Mullany, 2010).It is created by the layered growth of microorganisms existing as separated micro- colonies, mainly bacteria capable of adhering to each other, which can form a community where spatially dis- tributed populations can interact.When organized in biofilms, the oral micro-organisms are less susceptible to antimicrobials and more resistant to immunologi- cal defense systems (Davies, 2003; Dufour et al., 2013; Stoodley et al., 2002).Accumulation of biofilm microorganisms is very fast as a result of the interspecies aggregation of streptococci with actino- mycetes, as well as agglutination of microorganisms within one species, which leads to aggregation of new bacterial species with the already settled organisms.Among the early colonizers of the biofilm, different species of Streptococcus spp., Eikenella spp., Actinomyces spp., Haemophilus spp., Prevotella spp., Capnocytophaga spp., Priopionibacterium spp., and Veillonella spp.Immediately after adhesion of bacteria to the surface or to other bacteria, activation or inhibi- tion of specific gene expression occurs, with biofilm maturation being associated with changes in activity of particular genes in relation to the environmental con- ditions.This initial phase of adhesion becomes irrevers- ible later due to a specific reaction between bacteria adhesins and PRPs (proline-rich glycoproteins) on the surface of the acquired pellicle.Since microorganisms are not able to colonize the cleaned tooth surfaces, which are deprived of any exter- nal components, the presence of the acquired pellicle is necessary for adhesion.The biofilm matrix is increased by further 132 Struzycka I. 2 precipitation of salivary glycoproteins, mainly through the creation of extracellular disaccharides.One of the important func- tions of EPS, in addition to the significant role in the processes described above, is the protection of these microorganisms against the host defense system and this is especially meaningful for the pathogenic nature of cariogenic microorganisms.Over 700 bacterial species have been isolated from the human oral cavity and the majority of them are associated with dental biofilm.S. mutans produces a rare soluble fructan called inulin (Biswas et al., 2005; Takashi and Nyvad, 2008).Extracellular polysaccharides are created also by other bacteria in the oral cavity, such as S.sanguis and A. viscosus, but a major role in their production is attributed to S. mutans.After the adhesion phase, the process of building of the biofilm structure begins with microbial multipli- cation and differentiation (Belda et al., 2012, Filoche et al., 2010).The later colonizers include Fusobacterium nucleatum, Actinobacillus spp., Prevotella spp., Eubacterium spp., Treponema spp.Bacterial forms colonize the pel- licle acquired during the day, which is transformed into dental biofilm.The cariogenic streptococci form extra- cellular polymers in the enzymatic reactions involving ?-glucosidases.The coating made of pol- ysaccharides, due to its hydrophilic nature, effectively protects bacteria from phagocytosis (Allison, 2003).The sub-gingival microbiome is associated with gingivitis and periodontal disease (Abusleme et al., 2013).The composition of EPS varies depending on the bacterial strain and environmental conditions.Sticky mutan is involved in increasing of the plaque mass and enhances adhesion of the cariogenic microorganisms.From fructose, with involvement of ?-fructosidase, a polymer .called fructan or levan is derived.and Porphyromonas spp