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# SIGNIFICANCE OF BIOFILMS IN DENTISTRY

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## ABSTRACT

In the past decades significant scientific progress has taken place in the knowledge about biofilms. They constitute multilayer conglomerates of bacteria and fungi, surrounded by carbohydrates which they produce, as well as substances derived from saliva and gingival fluid. Modern techniques showed significant diversity of the biofilm environment and a system of microbial communication (quorum sensing), enhancing their survival.

At present it is believed that the majority of infections, particularly chronic with exacerbations, are a result of biofilm formation, particularly in the presence of biomaterials. It should be emphasised that penetration of antibiotics and other antimicrobial agents into deeper layers of a biofilm is poor, causing therapeutic problems and necessitating sometimes removal of the implant or prosthesis.

Biofilms play an increasing role in dentistry as a result of more and more broad use in dental practice of plastic and implantable materials. Biofilms are produced on the surfaces of teeth as dental plaque, in the paranasal sinuses, on prostheses, dental implants, as well as in waterlines of a dental unit, constituting a particular risk for severely immunocompromised patients. New methods of therapy and prevention of infections linked to biofilms are under development.

**Key words:** *biofilm, dentistry, dentures, implants, dental unit waterlines*

## INTRODUCTION

Microorganisms present inside or on external surfaces of a human body are referred to as a microbiome. It is calculated that a human body consists of about $10^{13}$ cells, while a cell count of a microbiome is 10-fold higher and amounts to approximately $10^{14}$ cells, which weigh around 1 – 2 kg (1).

Physiological microbial flora – apart from its many other functions – plays a significant role in protection of the host against pathogenic microorganisms, however sometimes itself may cause difficult to treat infections, particularly if they are associated with a biofilm (2). Presence of foreign bodies augments biofilm formation within a host (e.g. human). In dentistry it applies to – among others – dentures, obturator prostheses and dental implants.

Too little attention is being paid to a problem of biofilm formation within a dental unit waterlines and its link to etiology of infections in the oral cavity and systemic infections, particularly in immunocompromised patients.

## STRUCTURE AND FUNCTION OF BIOFILMS

Biofilms are multilayered accumulations of bacteria or fungi, consisting of one or many species of microorganisms (3, 4). These structures are common in the

external environment (e.g. aquatic reservoirs, sewage pipes, taps), but also inside the macroorganisms. A definition of biofilm which is valid at present describes it as a population of sedentary cells of microorganisms, irreversibly bound with the base and immersed in the matrix of extracellular polymeric substances (produced by these cells), showing a modified phenotype in relation to the pace of replication of bacteria and transcription of their genes (4).

In the process of biofilm formation, the earliest stage is adhesion of microbial cells to the surface, e.g. teeth or prostheses, as a result of interactions of superficial substances of the microorganism with components of saliva, which are present in so called acquired *pellicle* (covering the surface of the tooth enamel and necessary in the process of adhesion), or with substances contained in dental fluid (5-8). At first a reversible nonspecific interaction takes place between the microorganisms and abiotic material or live tissue, as a result of action of van der Waals, electrostatic and hydrofobic forces.

In the next stage a specific reaction takes place between the bacterial adhesins and the surface of the acquired *pellicle.* Close adherence of microbial cells to the underlying surface for a sufficient long period of time makes this bond irreversible (4). In the majority of cases the degree of adherence depends on microbial species and number of cells, speed of liquid flow, and physicochemical properties of a given surface. Subsequently microorganisms produce extracellular polymer substances (EPS) (3).

After the cells become irreversibly bound to the surface and produce extracellular polysaccharides, the speed and scope of the increase in the number of layers of the biofilm depend not only on the speed of liquid flow, but also on the content of nutrients, availability of iron, pH, osmolarity, oxygen content, concentration of antibacterial agents and ambient temperature (8). In the course of this process microcolonies are being formed and maturation of the biofilm ensues (fig. 1). An established biofilm may cause pathogenic process even in anatomically distant sites – as a result of breaking away of its fragments containing aggregates of bacterial cells, production of endotoxin, evasion of the immunological response of the host, as well as formation of a niche for replication of bacterial cells resistant to antimicrobials.

It is known at present that the structure and function of microorganisms in the biofilm may resemble multicellular organisms thanks to the interactions and communication between the cells, even belonging to different species. In the biofilm matrix they function as a consortium, cooperating in a relatively complicated and coordinated manner (4). Studies of the biofilm structure reveal its complexity – with a system of channels, which enables communication of the microbial cells and supplies them with nutrients and oxygen, removing at the same time waste products of their metabolism (9). Metabolic diversity of the microorganisms one be observed within the biofilm – cells forming deeper layers are metabolically less active than those in the layers closer to the biofilm surface (10). A system of communication between the microorganisms (quorum sensing) favours their persistence in the biofilm (6, 9). Intercellular communication, as well as the presence of extracellular substances, may influence the pace of bacterial growth, regulate expression of their genes, metabolic cooperation and competition of the cells, their physical contact and production of antimicrobial exoproducts (4, 7). Within the conglomerate there could be a change of expression of hundreds of genes. Research also shows that depending on the cell density in the biofilm, a coordination of activation of specific genes is being observed, which may lead to further increase of its volume (11).

Infections associated with biofilm formation are rarely eliminated by the host immune system. Despite

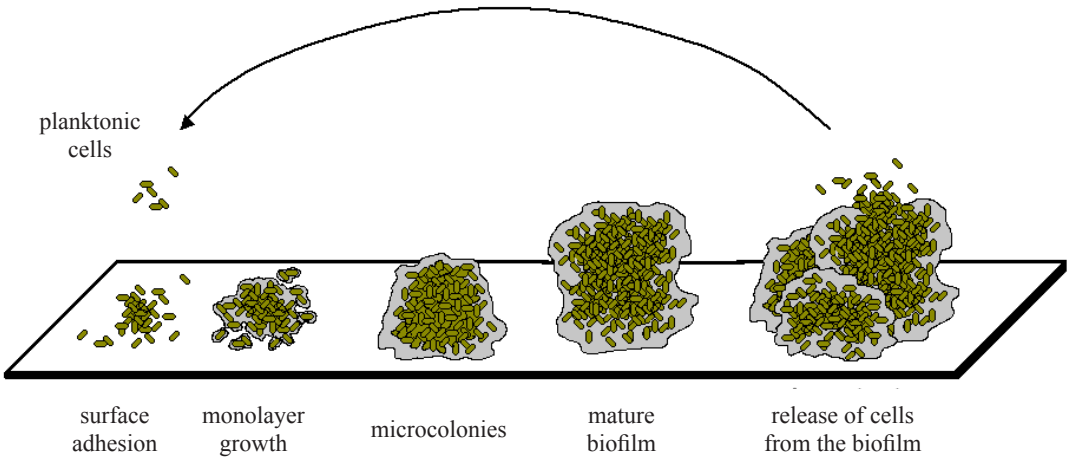


Fig. 1. Stages of biofilm formation

the fact that bacteria forming a biofilm release the antigens and stimulate antibody synthesis, they are protected against the immune response of the host, because the structure of the biofilm inhibits the access of antibodies, lysozyme, lactoferrin and granulocytes to the microorganisms forming it, what favours their persistence. Moreover, this immune response may even cause damage to the neighbouring tissues, as it favours maintenance of the inflammatory process (4, 12).

It is believed at present that majority of bacteria and fungi – if not all – may form a biofilm, however research indicates that several species, such as Gram-positive cocci *Staphylococcus epidermidis*, Gram-negative rod *Pseudomonas aeruginosa* and a yeast-like fungus *Candida parapsilosis*, are particularly prone to produce biofilms (9, 13, 14). This ability of bacteria and fungi to adhere to abiotic surfaces and produce a biofilm are at present considered as important virulence factors of these pathogens.

Some antibiotics work on the cells which are actively dividing, therefore the cells in the deeper layers of the biofilm – metabolically less active – may be more resistant to antimicrobial agents, as well as to detergents and disinfecting agents or antiseptics, in comparison to the cells closer to the biofilm surface or planktonic cells (15). It is also known that antibiotic penetration into the biofilm is limited or remnant, which causes problems in therapy of these infections. Results of many research studies indicate, that susceptibility of bacterial cells forming a biofilm to antibiotics is up to 1000-fold lower than of the cells outside of this structure (4, 16). Sometimes it necessitates removal of the implant or prosthesis. Other mechanisms responsible for resistance of bacteria to antibacterial agents within the biofilm are inactivation of the drug by extracellular polymers or enzymes modifying the antibiotics (4, 10).

At present it is believed that antibiotics may modify the function of the biofilm. Macrolides may play an important role in inactivation of the bacterial cells within the biofilm, as a result of their ability to inhibit quorum sensing, as well as due to immunomodulating activity of this group of agents, and it is already used therapeutically (17).

## CLINICAL SIGNIFICANCE OF BIOFILMS

At present it is calculated that biofilms are a cause of over 60% of all bacterial infections, particularly those with chronic course (6). They comprise infections associated with more and more common use of synthetic compounds in the form of central and peripheral vascular catheters, urinary catheters, valve system for *ventricular shunt,* stents or implants (4, 8). Biofilms are produced by microorganisms also on the surface or inside such structures as contact lenses, n*eedleless connectors, endotracheal tubes*, intrauterine devices, artificial heart valves, heart stimulators, peritoneal dialysis catheters, joint prostheses, tympanostomy tubes or laryngeal voice prostheses. Infections classified at present as associated with biofilm formation comprise also *inflammatory native* valvular heart disease, chronic bacterial prostatitis or infections linked to cystic fibrosis.

Biofilm formed inside the paranasal sinuses may be of great importance in dentistry due to anatomical proximity of these structures. Its presence is found on the surface of mucous membranes of 75-100% persons with chronic sinusitis (18, 19). Mladina et al. documented the presence of a biofilm in 62/65 (95.4%) of samples of „healthy" mucosa of the paranasal sinuses (20).

Infections associated with biofilm formation are characterised by recurrence of symptoms, even after several courses of antibiotics, since standard antibiotic therapy eliminates only planktonic (free floating) cells, while cells adhering to the surface are able to replicate within the biofilm and may continue to spread even after antibiotic therapy is completed (8).

## DENTAL BIOFILM

Dental plaque is the first – and until now the best – described biofilm in the human body. The newest molecular investigations indicate that it contains up to 1000 species of bacteria (21, 22).

There is diversification of bacterial flora on the tooth surface in the supragingival plaque in comparison to the subgingival plaque (tab. 1). In supragingival plaque predominate Gram-positive bacteria, while in subgingival plaque – mostly Gram-negative bacteria. Biofilm within the supragingival plaque plays a role in etiology of caries of the dental crown and root (so called caries of the root cementum) and caries at the margin of a restoration (so called secondary caries). Biofilm is also present in the infected root canal.

Tab. I. Bacterial flora in supragingival and subgingival plaque (21, 25, 51, 52)

| Supragingival plaque | Subgingival plaque |
|---|---|
| • *Streptococcus mutans*<br>Streptococcus salivarius<br>Streptococcus mitis<br>Streptococcus gordonii<br>• *Lactobacillus acidophilus*<br>• *Actinomyces odontolyticus*<br>Actinomyces israelii<br>Actinomyces naeslundii<br>• *Staphylococcus aureus*<br>• *Neisseria mucosa*<br>• *Capnocytophaga ochracea*<br>Capnocytophaga sputigena<br>• *Candida* spp. | • *Prevotella nigrescens*<br>Prevotella intermedia<br>• *Tannerella forsythia*<br>• *Fusobacterium nucleatum*<br>• *Campylobacter* spp.<br>• *Actinobacillus* spp.<br>• *Porphyromonas gingivalis*<br>• krętki<br>• *Synergistes* spp. |

Microorganisms being a component of a dental plaque, such as *Streptococcus mutans* and *Streptococcus sobrinus*, are considered as primary pathogens in etiology of the caries of enamel and root cementum, while rods of the genus *Lactobacillus* are responsible for disease progression (23). Other bacteria, including *Streptococcus oralis, Streptococcus milleri, Streptococcus salivarius*, as well as *Enterococcus faecalis, Actinomyces naeslundii* and *Actinomyces viscosus* probably may also cause – under favourable conditions – a dental caries (24). A comprehensive review article on this topic has been published recently (25).

Bacteria forming a biofilm of the subgingival plaque cause difficulties in therapy of gingivitis and periodontal disease. Nonspecific bacterial flora constitutes an inflammatory factor in gingivitis, while in periodontitis in gingival sulcus there are mainly bacteria such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia* and *Fusobacterium nucleatum* (26).

## DENTURE PLAQUE

A biofilm referred to as denture plaque constitutes a big clinical problem in dental practice due to a common use of dentures in a population (27). Large prosthetic restorations are usually made with acrylic compound, which creates favourable conditions for deposition of a denture plaque. It has been demonstrated that fungal cells adhere with ease to the surface of acrylic. In microscopic studies the surface of acrylic appears not uniform, with many porosities and denture plaque with *Candida albicans* cells penetrating into all indentations on the acrylic surface (28). Adhesion to acrylic compounds is particularly strong for mycelial form of fungi, due to penetration of hyphae and pseudohyphae into micropores of the acrylic. High humidity and the elevated temperature, lack of possibility of self-cleansing by saliva (particularly under the plate of the upper prosthesis) as well as poor hygiene of the prostheses, make easier the deposition of denture plaque as well as replication of bacteria and fungi.

Dentures constitute a surface which creates favourable conditions for biofilm formation by bacteria and fungi, and their density may reach up to $10^{11}$ cells/mg of denture plaque (29). They are most often formed by yeast-like fungi *Candida* spp., as well as bacteria of the genus *Streptococcus*, *Staphylococcus*, *Veillonella*, *Lactobacillus*, *Prevotella* and *Actinomyces* (30, 31). Prosthetic stomatopathies are usually caused by fungal infections of *Candida* spp. and by bacterial infections. Presence of bacterial and fungal biofilms is also detected on the surface of obturator prostheses (32).

In prophylaxis of infections it is of utmost importance to maintain good hygiene of the oral cavity and dentures by the wearers of prosthetic restorations. It should be emphasised that these biofilms may cause not only local infections in the oral cavity, but also dangerous systemic and generalised infections (33).

## IMPLANTS

Invention of dental implants constitutes an enormous progress in dentistry (7). Its significance continues to increase in esthetic dentistry, dental traumatology and in maxillofacial surgery. There is however a problem of biofilm formation on their surface and infections associated with it, which may even necessitate removal of the implant. Gram-negative rods characteristic for periodontal disease have been detected in peri-implant pockets affected by an inflammatory process. It should be noted that also other bacteria, such as *Staphylococcus* spp, enteric rods of the *Enterobacteriaceae* family and yeast-like fungi *Candida* spp., not linked etiologically to periodontitis, have also been present. Factors linked to a risk of biofilm formation on a dental implant is – among others – type of implant surface and presence of adhesion proteins produced by a given microorganism, as well as other local and systemic factors (34). In implantology materials are being used which are characterised by minimal microbial adhesion and biofilm formation (e.g. titanium and its alloys) (35).

## BIOFILMS IN DENTAL UNIT WATERLINES

Dental unit waterlines comprise a friendly environment for microorganisms forming a biofilm, particularly in case of water stagnation due to its infrequent use (e.g. during a break in dental office's hours). It has been documented that bacterial count in dental unit waterlines reaches $10^4 – 10^6$ colony forming units (CFU) per milliliter of water, while the recommended limit for nonsurgical dental procedures should be $\leq$500 CFU/ml (36). According to the American Dental Association guidelines, water in the dental unit waterlines should contain $\leq$200 CFU/ml (37).

A risk of infection associated with this bacterial flora is particularly high in immunocompromised patients who require dental treatment. Among bacteria isolated from water filling the dental unit waterlines very common are non-fermenting rods, such as *Pseudomonas aeruginosa* or *Acinetobacter baumannii*, often responsible for opportunistic infections in this group of patients (38). Mycobacteria and yeast-like fungi *Candida* spp. have also been isolated from dental unit water samples. *Legionella pneumophila* infection linked to dental treatment has been

reported recently (39). Bzdęga *et al*. recommend microbiological testing of water from dental unit waterlines twice a year, including testing for *Legionella* spp. (40). It has been documented that frequency of *Legionella pneumophila* presence in water from a dental unit may reach 25 – 36% of apparatuses (41, 42).

Several methods are being used in prophylaxis of infections associated with dental unit water, such as filters, chemical disinfection of waterlines (according to the manufacturer's recommendations) and the backwater valves (43). Dentists are also advised to flush waterlines of the dental unit for 2 – 5 minutes before starting work on a given day and for 30 seconds before using water in the next patient. Another method inhibiting a biofilm formation within dental unit waterlines could be their impregnation with antimicrobial agents, e.g. polyvinylidene difluoride (PVDF) (44).

## PERSPECTIVES FOR THERAPY OF BIOFILM-ASSOCIATED INFECTIONS

At present an intensive research is ongoing which aims at delineation of new options for therapy of infections associated with biofilms through a choice of appropriate antibiotic (e.g. a macrolide) or use of new antimicrobial substances, which are active within the biofilm. This group of agents comprises cationic proteins, cationic monomers, quorum sensing inhibitors (QSIs) and substances "dissolving" biofilms (45-47). Special hope is linked to development of broad spectrum substances, which could be used in patients – e.g. at present NVC-422 (N, N-dichloro-2, 2-dimethyltaurine) is under clinical trials. The results of the newest research show that infections associated with biofilms may be treated thanks to synergistic effect of antibiotics combined with other substances, such as mucolytic agent N-acetylcysteine, ethanol or EDTA (48). Moreover, biofilms produced by *Candida albicans* may be treated with a combination of an antifungal agent (e.g. amphotericin B, caspofungin or fluconazole) with an antibiotic active against Gram-positive microorganisms, such as doxycycline or tigecycline (48, 49).

There is at present a trend in dental implantology to use materials with dual function – inhibiting microbial adhesion, while at the same time stimulating integration of the implant with surrounding tissues, e.g. due to amino acid content, such as arginine, glycine and asparaginic acid (50).

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