REVIEW ARTICLE

Antibiotic resistance: a "dark side" of biofilm-associated chronic infections

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KEY WORDS

antibiotic resistance, bacterial biofilms, chronic infections, dental plaque, periodontitis

ABSTRACT

Bacteria may exist in nature in a planktonic form or in biofilms that allow bacteria to survive in an unfriendly microenvironment. Biofilm is a structured community of bacteria hidden in a self-produced polymeric matrix of polysaccharides, proteins, and extracellular DNA. Biofilm-growing bacteria cause chronic infections, which are characterized by persisting inflammation and tissue damage (chronic rhinosinusitis, chronic wounds, periodontal diseases). Importantly, some bacteria of human microbiome, when growing in a biofilm (e.g., *Porphyromonas gingivalis* in dental plaque), can become destructive and can contribute to an association between local infections (periodontitis) and systemic diseases such as atherosclerosis or rheumatoid arthritis. The biggest clinical challenge with biofilm-associated infections is their high resistance to antibiotic therapy. Therefore, biofilm formation should be prevented either by antibiotic prophylaxis or early aggressive pharmacological therapy. In this review, we also discuss novel antibiofilm therapeutic strategies based on compounds that can destroy the biofilm matrix and increase susceptibility of biofilm-forming bacteria to antibiotics and host defense system.

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Received: June 5, 2013.
Accepted: June 6, 2013.
Conflict of interest: none declared.
Pol Arch Med Wewn. 2013; 123 (6): 309-313

Introduction Modern microbiology has transformed our understanding of the role of microbial biofilms in the pathogenesis and treatment of a variety of chronic infections. Biofilms are recognized as a major cause of persistent infections and chronic tissue-destructive inflammatory diseases.[1] For years, formation of biofilm has been studied on foreign surfaces such as intravenous catheters, orthopedic and stomatology implants, and other biomaterials relevant to the development of device-associated infections. Today, it is commonly accepted that the majority of chronic bacterial infections are characterized by biofilm formation on natural surfaces. Biofilm growth occurs on hard or soft tooth surfaces (dental plaque), heart valves, mucosal epithelial cells, and skin cells.[2] Importantly, pathogens enclosed in the biofilm matrix are resistant to antibiotics and host defense. Therefore, biofilm-associated infections persist despite targeted antibiotic therapy and activation of the host immune defense. It raises serious therapeutic problems in human and veterinary medicine.[2-4]

Mechanisms of biofilm formation In nature, bacteria exist in two forms: planktonic (free-floating cells) and biofilm, that is, consortia of microorganisms (sessile cells) adhering to biological and nonbiological surfaces. This adaptation, a switch from planktonic to the biofilm mode of growth, has been implicated as a survival strategy, common for microorganisms living in an unfriendly environment. Importantly, both human microbiome bacteria and pathogens can form biofilms on various surfaces including mucosal membranes, teeth, and medical devices. More than 80% of chronic bacterial infections are associated with biofilm.[1] Microbes form a biofilm in response to various factors, which may include the recognition of attachment sites on a surface. Most importantly, biofilm formation is induced by exposure of planktonic cells of pathogens to subinhibitory concentration of antibiotics.[5]

Bacterial biofilm formation is a dynamic process. It begins with the attachment of planktonic bacteria to a surface (e.g., at a gate of infection). If the colonists are not separated from the surface, they can anchor themselves more permanently.

Then bacteria multiply and produce extracellular polymeric substances (EPS), components of the biofilm matrix. The composition of EPS varies depending on the bacterial strain and environmental conditions (e.g., contact with inflammatory cells), but, in general, EPS consists of exopolysaccharides, proteins, and extracellular DNA (of bacterial or neutrophil origin).[6,7] Biofilm matrix immobilizes bacteria and traps nutrients and various biologically active molecules, such as bacterial communication signals generated by the quorum-sensing systems.[8] Moreover, the matrix acts as a shield against antimicrobials, toxins, and antibodies. However, it is unclear whether EPS molecules can be effectively recognized by toll-like receptors or any receptor of innate immunity.

The entire life cycle of a biofilm consists of several stages: initial attachment (planktonic cells adherence), irreversible attachment, maturation, and dispersion. Dispersal of cells from the mature biofilm and their migration to new surfaces enable biofilms to spread and are responsible for transition of infections from local to systemic ones.[9]

Clinically important sites of biofilms As mentioned above, in nature, most bacteria exist in biofilms, including bacteria of human microbiome, especially normal flora bacteria of the mouth, skin, vagina, and gut.[10,11] Furthermore, biofilms are found in the most clinically important sites for infections. The following chronic infections have been found to be associated with biofilm formation (bacterial, fungal, or polymicrobial biofilms): acne vulgaris, breast implant infection, burn-related infection, chronic sinusitis, central nervous system shunt infection, chronic otitis media, dental implant infection, intravascular and peritoneal dialysis catheter infection, lung infection in cystic fibrosis, periodontitis, prosthetic joint infection, urinary stent infection, chronic wounds (wounds that fail to heal), and others.[10-13]

In the next part of this review, we will focus on clinical implications of the oral biofilm, one of the best-understood microbial communities associated with the human body.

Links between oral biofilms, periodontal infections, and systemic diseases Oral biofilm, or dental plaque, accumulates through a sequential colonization of oral surfaces (dental hard and soft tissues) by the different species present in the oral cavity.[14,15] Over 700 bacterial species have been isolated from the human oral cavity and the majority of them are associated with dental plaque. Supragingival plaque is dominated by gram-positive bacteria, including *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, and lactobacilli, while the subgingival plaque is dominated by gram-negative anaerobic bacteria, such as *Actinobacilllus*, *Campylobacter spp*., *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*.[12,14,15] It has also been well documented that bacteria of oral biofilms formed on dental hard and soft tissues as well as on different biomaterials (e.g., tooth implants, orthodontic appliances) are responsible for major oral diseases: dental caries (tooth decay) and periodontal inflammatory diseases (gingivitis and periodontitis). Although the initiating factors that lead to disease development are not clearly defined, the key role of *Porphyromonas gingivalis* in a shift from a health-associated to pathogenic biofilm community has been well documented.[16] Importantly, a number of clinical studies have shown that *Porphyromonas gingivalisis* is not only involved in the pathogenesis of periodontal diseases but also in systemic diseases related to periodontal infections. Patients with severe chronic periodontitis have been reported to have a significantly increased risk of cardiovascular diseases, including atherosclerosis, myocardial infarction, and stroke.[17] Moreover, a remarkable association between periodontal diseases and other disorders, such as diabetes, adverse pregnancy outcome, and rheumatoid arthritis, has been demonstrated.[18-20] From these data, it is clear that oral infections may represent a significant risk factor for systemic diseases. Therefore, the control of oral biofilm growth before the development of oral infections is essential for the prevention of these systemic conditions. An early prevention is extremely important because eradication of pathogenic bacteria from a mature biofilm may be a serious therapeutic challenge due to common biofilm resistance to antimicrobial agents.

Biofilm resistance to antibiotics and to host defense mechanisms The biological properties of bacterial cells of mature biofilm differ from those of planktonic cells of the same bacterial strain. Importantly, these acquired properties allow biofilm-hidden bacteria to survive in nature, especially in unfriendly microenvironments. This adaptation has significant diagnostic and therapeutic consequences.[1,21]

Biofilm-growing bacteria exhibit increased resistance to antibiotics and disinfectants. The effective therapeutic concentration of some antibiotics to bacteria in biofilm may be even 100- to 1000-fold higher than that to planktonic bacteria.[2] Moreover, bacterial biofilms are resistant to phagocytosis and other mechanisms of innate and adaptive immune system.[21,22] Accordingly, biofilm-growing pathogens cause persisting infections and chronic tissue-destructive inflammation.[10]

Resistance to antimicrobial agents is the most important cause of noneffective therapy of biofilm-associated infections, and, importantly, it is multifactorial. Results of the recent studies have suggested which mechanisms are responsible for resistance to antibiotics.[21,23] First, biofilm growth is associated with an increased number of mutations leading to generation of antibiotic-resistant phenotypes of bacteria, and genes involved in antibiotic resistance are correlated with biofilm phenotype.[24] Second, the production of

the exopolysaccharide matrix contributes to an increased cell survival by slowing down antimicrobial diffusion speed. Third, differences in bacterial density throughout the biofilm determine gradients of nutrient and oxygen availability, resulting in the differences in metabolic activity among bacteria. It has been proposed that slow-growing and nongrowing bacteria contribute to increased biofilm resistance to antibiotics.[25] Finally, the upregulation of efflux pomp proteins and activation of quorum-sensing systems reduces and neutralizes incoming antimicrobial agents.

Importantly, a number of studies have shown that subminimal inhibitory concentrations of some antibiotics can induce biofilm formation in vitro, a process that may have clinical relevance. The majority of well-documented studies investigating the mechanisms of antibiotic-induced biofilm formation have been performed using the common device-associated pathogens, including *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa*.[5]

It is tempting to speculate that such unwanted biofilm induction may occur during the course of an antimicrobial antibiotic therapy due to varying gradients of systemic antibiotic concentrations, especially at the beginning of treatment. Therefore, to decrease a risk of biofilm induction, we should start with high doses of chemotherapeutics from the very beginning of a diagnosed infection.

In addition, except for the well-documented biofilm resistance to a variety of antimicrobial agents, an increasing number of studies have suggested impaired host defense mechanisms against biofilm-form of bacteria, especially those dependent on neutrophil activity.

Leid et al.[26] suggested that biofilm resistance mechanisms to phagocyte killing and other innate defense mechanisms may consist of limited penetration of immune cells (neutrophils) and their antimicrobial products (ROS) into the biofilm; inactivation or suppression of leukocyte-specific processes by biofilm components (matrix or cell components); decreased ability of leukocytes to phagocytize biofilm bacteria; quorum-sensing molecules that increase resistance to leukocytes in biofilm; and genetic switches that lead to the generation of bacteria phenotypes, which are more resistant to the defense system components.

Nevertheless, it is not yet well understood how the immune system reacts with biofilm. Further studies are necessary to examine different bacterial biofilms because the interactions between bacteria and leukocytes seem to be pathogen-specific. For example, it has been shown that neutrophils were able to phagocytize *Staphylococcus aureus* biofilms,[7] while other authors reported that neutrophils were immobilized on biofilms of *Pseudomonas aeruginosa*; thus, their killing capacity was limited.[27] In addition, it has been shown that exopolysaccharide alginate protects *Pseudomonas aeruginosa* biofilm bacteria from interferon-γ-mediated macrophage killing.[26] All these results suggest that biofilm-associated neutrophils, similarly to previously described cancer-associated neutrophils, do not play a beneficial role in host defense.

Conclusions: new strategies in prevention and treatment of biofilm-associated infections Chronic biofilm-associated infections that do not respond to antibiotic therapy are a serious clinical challenge in human and veterinary medicine. To overcome these difficulties, new strategies for treatment of microbial biofilms have been recently proposed. Clearly, there is a need for novel biofilm-targeted therapies that are designed to neutralize/eliminate the mechanisms responsible for biofilm resistance to antibiotics and antiseptics.

To facilitate antibiotic diffusion into biofilms, we need agents that will be able to penetrate and destroy the components of biofilm matrix and kill hidden bacteria. Such antibiofilm drugs should be applied either before or along with antibiotics. The most promising candidates for such strategy are DNase,[28] lactoferrin,[29] chlorhexidine ("the golden standard" in stomatology),[30] taurolidine,[31] and antiexopolysacharide agents.[32] The results from our studies suggest that taurine haloamines, especially taurine bromamine (TauBr), is a promising candidate for treatment of biofilm-associated infections. TauBr shows antimicrobial and anti-inflammatory properties. In vitro, it effectively kills a variety of pathogens. Recently, we have reported that TauBr is able to inhibit in-vitro formation of *P. aeruginosa* biofilm but alone cannot destroy the mature biofilm and kill sessile bacterial cells.[33,34] However, clinical studies have shown that TauBr is effective in local treatment of acne vulgaris, a biofilm-related inflammatory skin disease.[35] Another novel antibiofilm therapeutic strategy suggests using quorum-sensing inhibitors to block an intercellular communication between biofilm-sessile bacteria and, therefore, to interfere in a biofilm life-cycle.[5,36]

In conclusion, our increasing knowledge about the nature and mechanisms of growing biofilm is crucial for efficient prophylaxis and treatment of chronic biofilm infections. The development of novel cotherapeutic agents may help guide antibiotic therapy and diminish antibiotic resistance, an old challenge in human and veterinary medicine. Importantly, as mentioned above, to avoid biofilm formation, we need to use high concentrations of antibiotics from the very beginning of therapy. Moreover, the entire biofilm matrix should be removed from body surfaces to avoid biofilm recurrence.

Acknowledgments We thank Maria Walczewska for technical assistance in the preparation of this manuscript for publication. This paper was supported by a grant from the Jagiellonian University Medical College (K/ZDS/002 964).

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ARTYKUŁ POGLĄDOWY

Antybiotykooporność – „ciemna strona" biofilmu towarzyszącego przewlekłym infekcjom

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antybiotykooporność, biofilm bakteryjny, choroby przyzębia, płytka nazębna, przewlekłe infekcje

STRESZCZENIE

Bakterie mogą występować w naturze w formie planktonicznej lub w formie biofilmu, który umożliwia bakteriom przetrwanie w nieprzyjaznym mikrośrodowisku. Biofilm to uporządkowana społeczność bakterii ukryta w polimerycznej macierzy własnej produkcji, składającej się z polisacharydów, białek i zewnątrzkomórkowego DNA. Rozwijające się w biofilmie bakterie powodują przewlekłe infekcje, charakteryzujące się przewlekłym zapaleniem i niszczeniem tkanek (przewlekłe zapalenie zatok przynosowych, niegojące się rany, choroby przyzębia). Co ważne, niektóre bakterie ludzkiego mikrobiomu, wzrastając w biofilmie (np. *Porphyromonas gingivalis* w płytce nazębnej) są przyczyną przewlekłych stanów zapalnych (*periodontitis*) i mogą stać się ważnym ogniwem łączącym miejscowe zapalenie z chorobami ogólnoustrojowymi, takimi jak miażdżyca i reumatoidalne zapalenie stawów. Największym klinicznym problemem związanym z zakażeniem z towarzyszącym biofilmem są trudności terapeutyczne wynikające z dużej oporności bakterii na antybiotyki. Zatem, tworzenie biofilmu powinno być powstrzymane albo na etapie profilaktyki antybiotykowej lub wczesnego agresywnego leczenia farmakologicznego. Artykuł porusza także kwestię nowych strategii leczniczych przeciwdziałających tworzeniu się biofilmu, opartych na związkach chemicznych, które są w stanie niszczyć macierz biofilmu, a także zwiększać podatność bakterii tworzących biofilm na działanie antybiotyków i układu odpornościowego gospodarza.

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Praca wpłynęła: 05.06.2013.
Przyjęta do druku: 06.06.2013.
Nie zgłoszono sprzeczności interesów.
Pol Arch Med Wewn. 2013; 123 (6): 309-313