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Biofilm Inhibition: Compounds with Antibacterial Effects

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ABSTRACT

Biofilms can form on living or inert surfaces and prevail in natural, industrial, and hospital environments. They are made of bacteria organized in a coordinated functional community. Biofilms do not respond to antibiotic treatment due to multiple mechanisms of tolerance and resistance. If bacteria are coordinated in a biofilm form, they are significantly less susceptible to antibiotics, thus making the therapeutic approach difficult. The possibility of using drugs aimed at inhibiting the formation of biofilms in combination with current antibiotics is a therapeutic approach with a major potential for this type of persistent bacterial infection. This bibliographic study aims to present the main compounds that act by inhibiting or destroying the bacterial biofilm.

Keywords: biofilm inhibition, bacterial infection, polysaccharides

INTRODUCTION

In recent years, concerns have been raised about the surgical failures of an increasing number of joint replacement techniques. Some of these failures can be attributed to infectious complications. Skin bacteria can lead to infections related to the biomaterials used in these surgeries. It has been described in the literature that approximately 70% of nosocomial infections are caused during or after the implantation of an artificial medical device. The treatment of these acquired nosocomial infections is extremely meticulous and difficult, requiring an interdisciplinary team with a modern and evidence-based approach.[1] The purpose of this bibliographic study was to evaluate, describe, and compare data from the current literature on the most commonly used compounds for inhibiting biofilm formation.

BIOFILM FORMATION

Biofilm represents a group of microorganisms in which the cells stick to each other and to a surface. The adherent cells are embedded in a thin extra-cellular matrix

composed of polymeric substances.[2] Biofilm formation is one of the many ways for microorganisms to adapt and survive, especially in the environment.[3] After formation of the biofilm, microorganisms are able to increase their resistance to antibiotics and to the body's natural immune response, resulting in difficulties in treating the diseases they cause.[3] In human medicine, the formation of biofilms can lead to fatal complications, as they form on the interfaces of implantable devices such as catheters, heart valves, orthopedic prostheses, or intrauterine devices.[4,5]

IN VITRO VERSUS IN VIVO PROPERTIES

In vivo, the response of the human body and human cells to biofilm formation differs from the in vitro situation due to the immense variability of existing biological variables, the complexity and co-occurrence of various biological phenomena, the presence of the immune system, and the differences in the oxygen pressure of different compartments in which biofilms are formed.[6] Infections caused by *Pseudomonas aeruginosa* have received increased attention over the last decades and are currently the most studied infections in the literature.[7]

As mentioned above, there are major differences in the ways bacteria form, grow, and develop according to the environment. Although in vitro bacterial cultivation offers certain advantages in terms of growth, control, and proper analysis of variables, it has been shown that in vivo, certain oxygen-deficient areas favor biofilm formation, largely due to the presence of polymorphonuclear cells and the existing physiological conditions.[7]

For the human species, several locations of bacterial infections that involve biofilms associated with oxygen deficiency have been described, namely:

- sputum from cystic fibrosis;
- sinus secretions from cystic fibrosis;
- in the scar tissue or in the bone.

It is important to note that in vivo, there is currently no qualitative or quantitative evaluation of the amount of antibiotic present at a given time in the biofilm.[8] In these oxygen-poor areas, the actions of certain bactericidal compounds (beta-lactam, aminoglycosides) are also inhibited, and the transport of antibacterial constituents is dependent on transmembrane oxygen transport. This latter phenomenon has been more closely followed in the case of aminoglycosides.[9]

There are authors who described biofilms as third compartments, blood being the primary compartment and tissues the secondary compartment. Therefore, as the antibiotic reaches the biofilm, it is considered necessary for it to have passed through the primary and secondary compartments.[10] Experimental models that mimic bacterial behavior in vivo have been described in the past and are currently being used in some innovative projects. The most widely used and easiest experimental model, described by Rupp *et al*. in 1999[11] and by Kadurugamuwa *et al*. in 2003,[12] involves inserting a plastic catheter into the dorsal skin tissue of mice. This model involves biofilm formation in vitro prior to implantation in experimental animals and almost mimics the behavior of an infected catheter. The major problem with artificially created biofilms is that the inoculum used contains an excessive amount of bacteria that does not normally appear in nosocomial infections.

There are models of endocarditis induced by bacterial inoculum, but they need more specialized teams, special tools, and larger animals (e.g., rats or rabbits), for which authorizations and approvals by ethics committees are difficult to obtain.[13]

Certain authors have attempted to mimic wound infection associated with biofilms. The skin of animals authorized for scientific research is different from human skin; thus, the results are not conclusive. However, in 2012, Roche *et al*. have succeeded in mimicking infections associated with biofilms on porcine skin, by using Methicillin-resistant *Staphylococcus aureus* – MRSA.[14]

Models that attempted mimicking biofilm infections from cystic fibrosis faced two major impediments:

- infection with the PAO1 strain of *Pseudomonas aeruginosa* is most commonly used to create experimental models, but it mimics an acute, not chronic infection as described in cystic fibrosis;
- the bacteria were grown in vitro in a culture medium containing agar, with the main purpose of slowing down mechanical cleaning.[15]

INNOVATIVE AGENTS THAT INHIBIT THE FORMATION OF THE BIOFILM

The current trend in bactericidal and bacteriostatic therapy is directed to many unconventional methods of treatment supported by evidence-based medical research. Among the most innovative non-conventional methods for the development of new antibiotics are the bacterial adhesion and biofilm formation process, as well as their control system, the sensitive quorum system. The sensitive quorum system is a bacterial communication mechanism used to coordinate bacterial activities.

TABLE 1. Types of bacteria used and the corresponding ATCC code, according to the study conducted by de Lima Pimenta *et al.*[16]

Bacteria	American Type Culture Collection (ATCC) Code
Enterococcus faecalis	19433
Pseudomonas aeruginosa	27853
Staphylococcus aureus	25923
Staphylococcus epidermidis	35547
Streptococcus mutans	25175

A study published in 2013 by de Lima Pimenta *et al.* tested several compounds with potential biofilm inhibitory effect, as well as the bacterial communication mechanism and the sensitive quorum system.[16] Five main compounds were identified and studied, basically various gallic acid alkyl-esters (GEt, GHex, GOctad, G19, and C33). The species of bacteria that have been tested are presented in Table 1.[16]

Methyl galate (GMet) was the only derivative that exhibited biofilm activity of all bacterial strains evaluated, with an inhibitory activity of 91%.[16] Another study has shown that the injection of peptide-inhibitor-RNA III in rats with MRSA infections caused suppression of the protein-activating RNA III and of the susceptible quorum system necessary for biofilm formation.[17] Furthermore, in one experiment, a natural metabolite of lichens, called 6-gingerol, interfered with the formation of a sensitive quorum system, leading to the inhibition of *Staphylococcus aureus* biofilm formation, and changed the morphology of biofilms formed by *Pseudomonas aeruginosa*.[18]

In a bibliographic study from 2015, several compounds have been described to interfere with the formation of the sensitive quorum system, including penicillanic acid, solenopsin A, catechin, ellagic acid derivatives, and curcumin.[19]

Penicillanic acid is a major penicillin degradation product that has been shown to have a quorum inhibitory effect, being particularly effective on biofilms formed by *Pseudomonas aeruginosa*.[20] In another study involving *Pseudomonas aeruginosa*, phenyl-4,5-dihydroxy-2,3-pentanedione in combination with gentamicin demonstrated inhibitory effects on both the quorum system and pyocyanin formation, which could play a role in the dispersion or maturation of biofilms.[21]

The effect of quorum-sensing inhibitors has been analyzed in a current research, which studied the influence of tobramycin, in combination with clindamycin and vancomycin, with and without the addition of sensitive quorum inhibitors, namely cinemaldehyde, hamamelitin, and baicalin hydrate, on the activity of *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. The use of antibiotics, with the addition of susceptible quorum system inhibitors, has increased the potency of the bactericidal action of vancomycin, clindamycin, and tobramycin.[22]

The action of balcalein and 14-alpha-lipoyl-andrographolide was tested on *Pseudomonas aeruginosa* biofilms. The two compounds showed synergistic activity on biofilm inhibition and inhibitory activity on the sensitive quorum. However, the clinical relevance of these compounds is questionable, because the studies were conducted in vitro.[23]

A garlic extract named ajoene has been studied on *Pseudomonas aeruginosa* strains, demonstrating an inhibitory effect on virulent factors of the sensitive quorum (e.g., rhamnolipids). In the same study, ajoene had a synergistic effect with tobramycin and toxic effects on *P. aeruginosa* biofilms, and demonstrated lithic effects on polymorphonuclear cells.[24,25]

Ajoene was also observed in a recent study to demonstrate inhibitory and mediating capacity of the quorum system by lowering sRNA expression. This compound is considered the first and only able to mediate and regulate the activity of sRNA expression, and at the same time to have inhibitory effects on the quorum system, on both Gram-negative and Gram-positive bacteria. The compound is considered a new weapon in the treatment of cystic fibrosis, being potentiality feasible to be administered in association with the above mentioned antibiotics.[26]

Polysaccharides are a class of compounds existing in the extracellular matrix that forms biofilms. Several polysaccharide compounds extracted from bacteria or plants with inhibitory capacity on biofilm formation have been reported in the literature.[27]

Recent studies show that there are certain exogenous polysaccharides that act as inhibitors of biofilm formation. A recently studied compound is polysaccharide EPS 273, extracted from marine bacteria (*P. stutzeri*), which reduces the formation of *P. aeruginosa* biofilms. The main targets of this compound are the virulent factors of the biofilm and exoproteases. This compound also interferes with pyocyanin formation, subsequently inhibiting complex biochemical processes by forming stable biofilms at certain substrates. It is believed that this compound can be studied in perspective and even be used in the food and medical industry to inhibit the formation and multiplication of pyocyanic bacillus.[28]

Other polysaccharides with inhibitory action on biofilms are Ps1 and Pel, which decrease the capacity of *Staphylococcus epidermidis* biofilm formation in vitro. In

conclusion, polysaccharides can be used as an adjuvant with available antibiotics, by reducing their minimal concentration, to eradicate biofilm by reducing the chances of medical device-related infections.[28]

COMPOUNDS WITH UNKNOWN MECHANISM OF ACTION

There are studies reporting spectacular biofilm inhibitory effects, but the nature of inhibition is not yet elucidated. Esculetine, a coumarin derivative, is considered to have biofilm inhibitory activities but does not possess a clearly described mechanism of action. It is known that the thickness of a mature biofilm is reduced in the presence of esculetine. Another compound, derived from plants, with the role of food colorant, fisetin, is known for its inhibitory effects on the growth of biofilm thickness and maturation. Octenidine is another compound with inhibitory effect on biofilm, but its mode of action is also not clear.[29]

CLINICAL ASPECTS OF BIOFILMS

The first step in detecting and managing an infection with the potential for biofilm formation is the proper detection of the infection present in the human body. Multiple anamnestic, clinical, and laboratory details can guide the clinician to a precise diagnosis, and personalized and effective treatment. The analysis of biofilm fragments can be extremely difficult with classical methods in agar or classic bacterial cultures.[30]

In order to determine the biofilm infection, it is therefore necessary to examine by direct microscopy or by molecular determination, which is not widely available. There are certain antibodies and antigens that have been studied for diagnosis. The polysaccharide adsein is a highly expressed compound in the biofilm of cells, and was proposed in 2002 as the primary target of polysaccharide anti-adhesin antibodies, to be used for the detection and monitoring of staphylococcal infections in patients with vascular grafts or orthopedic implants.[31]

The Parsek-Singh biofilm criteria[31] were adopted by clinicians to track biofilm activity and include the following:

- the bacteria is adhered or associated with a substrate;
- direct microscopy examination presents areas with bacteria in groups wrapped in constituents of the host;
- the infection is localized;
- the infection is resistant to antibiotic treatment despite sensitive antibiotics;

In the future, we expect simple and minimally invasive biofilm detection, in a clinical setting, with easier management of infections that are resistant to classical treatments. The positive detection of a clinical biofilm would prompt physicians to immediately use therapeutic approaches and antibiotic combinations appropriate for persistent biofilm-related infections.

CONCLUSIONS

Biofilms and how they form and act is an actual problem even today, which is hard to define and clarify. Agents used to inhibit or destroy biofilms are growing, with new discoveries that can bring innovative insights. From a clinical point of view, an interdisciplinary approach is needed to eradicate an infection associated with biofilms.

CONFLICT OF INTEREST

Nothing to declare.

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