Microreviews in Cell and Molecular Biology 2012

# Biofilms in Cystic Fibrosis Patients

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**Key Words:**
Cystic fibrosis, *P. aeruginosa*, treatments, antibiotic resistance

**Cystic fibrosis (CF) is a genetic disease that causes infection in the lungs. The lung infection is cause by a buildup of bacterial biofilms. This results in blockage of the airways of the patients. A bacterial biofilm has a unique ability called horizontal gene transfer that increases the antibiotic resistances; this is a problem for many antibiotics and current treatments. Biofilms in cystic fibrosis patients are becoming categorized as polymicrobial; this means the biofilm contains more than one type of microorganism. One specific organism *P. aeruginosa* has been linked to increased antibiotic resistance. New technology was developed to help advance our knowledge of biofilm development. Research has been done to show the effectiveness of specific antibiotics and the effect certain microorganisms have on biofilm growth. Continuous use of antibiotics could result in organisms developing resistance.**

## Introduction

Cystic fibrosis (CF) is a genetic disease that causes mucus to build up in the lungs, which leads to bacterial infection of the airways. It is caused by a mutation in the CF transmembrane conductance regulator (CFTR). It is one of the most common inherited lethal genetic diseases. [1] Biofilms play a key role in the development of lung infections in CF patients. They build up in the patients lungs until the airway is completely blocked. A biofilm is a biopolymer matrix that provides structural stability and protection. [2] To penetrate the biofilm protection, large amounts of antibiotics are required. The polymicrobial biofilms found in CF patients are antibiotic resistant due to the large amount of antibiotics they are exposed to during the course of treatment. [3] One specific organism *Pseudomonas aeruginosa* is the target for most antibiotics for CF patents. [3] The presence of the organism causes more frequent hospitalizations and more rapid deaths than the other microorganisms. [1] *P. aeruginosa* is also responsible for many other types of infections in animals.

The accumulation of polymicrobial biofilms greatly increases the antibiotic resistance of the all the microorganisms in the biofilm due to horizontal gene transmission. Horizontal gene transmission allows the transfer of specific genetic information that increases antibiotic resistance of the organisms. The mature biofilms have the maximum tolerance of antibiotics; patients with biofilm infections should get treatment within the early stages of development. [2] Once the biofilm is formed there is little chance of the penetrating it. Biofilms can have different antibiotic resistance depending on the microorganisms. *P. aeruginosa* has the highest antibiotic resistance because of hypermutable phenotype, meaning that the species of microorganism has a higher mutation rate. [4] Monospecies biofilms containing *P. aeruginosa* experimentally have been shown to be most resistant to clinical antibiotics. Mixed species biofilms with *P. aeruginosa* have been shown to contain the same antibiotic resistance as the monospecies biofilm of *P. aeruginosa*. [3]

## Recent Progress

Very little is known about the organisms that are the main cause of lung infection in CF patients. Using a new technique called the Biofilm Ring Test, they were able to find that one chemical agent, clarithromycin, used in treatment had no effect on the biofilm production of *P. aeruginosa*. Combinations of antibiotics, that included clarithromycin, were shown to have an effect on the mature polymicrobial biofilm. The Biofilm ring test uses magnetic beads that become embedded as the biofilm is growing; this allows researchers to extract data on the growing biofilms. [1] Adding the magnetic beads into the biofilm has to occur during the beginning phases of

 

biofilm growth. A 12-day-old-biofilm was more resistant to a combination antibiotic treatment than a 24-h-old.[3] In the mature stages of the biofilm, it cannot be penetrated. Researchers have found that antibiotics cannot penetrate the biofilm, unless the microorganisms dispersed the biofilm. [5]The only access to the microorganisms inside is at the very beginning of biofilm formation.

*P. aeruginosa* was found to create the most biomass in mixed biofilms. [3] In mixed biofilms with *P. aeruginosa*, the minimum biofilm eradication concentration (MBEC) for all of the antibiotics used was the same as the MBEC concentration as the single species biofilm of *P. aeruginosa*. [3] This organism is the cause of higher antibiotic resistance in the biofilms than any other organisms, which is why *P. aeruginosa* is the target for many antibiotics. The current method to prevent chronic *P. aeruginosa* biofilms in the lungs of CF patients is aggressive hygienic measures to prevent cross contamination. [2] Hygiene routines have not proven to be an effective method to prevent the infection of *P. aeruginosa*. Once the organism is in the patients' airways, it is not removed.

Recently it was discovered the ability of *P. aeruginosa* to become mutated or hypermutated. The hypermutated phenotype of CF *P. aeruginosa* is the result of an alteration in the genes of one of two repair systems in the cell. Oxidative stress is considered to enhance mutability in biofilms. [2] The natural strains of *P. aeruginosa* have not shown any ability to hypermutate. The mutation only occurs in the lungs of the CF patients and in other parts of the body. [4] Researchers are not sure what is causing these mutations, further studies have speculated that the mutations could be cause by the state of the patients lungs, due to treatment or the existing organisms in the lungs. [4]

There is an array of microorganisms in the biofilm blocking the airways of CF patients. This is one of the problems for trying to eradicate biofilms in patients. Azithromycin is an antibiotic used for CF patients and it is shown to block quorum sensing in *P. aeruginosa*. The only side effect is that there has been increased antibiotic resistance in another type of microorganism in the lungs of the CF patient. [2]The current treatment for cystic fibrosis includes; Combinations suppressor therapy and DNase inhaled everyday [2]. Both treatments are continued for the rest of the patient's life.

**Discussion**

There is not a lot of information about the organisms in polymicrobial biofilms inhabiting the lungs of cystic fibrosis patients. Each organism needs to be studied so that a better target can be specified for antibiotic targeting. Knowledge is power. One organism, *P.aeruginosa*, is the antibiotic target. It is an effective target because it has the highest antibiotic resistance but that does not take into consideration the other microorganisms. There is an antibiotic used for treatment that is being used to break down biofilms containing *P.aeruginosa* but it increase antibiotic resistance in othermicroorganisms. A more broad approach for antibiotics could eliminate all of the organisms and reduce the potential for development of antibiotic resistance.

Techniques and mechanisms should be developed so the interactions between microorganisms in biofilms can be studied. In one of the studies the new test called the Biofilm Ring Tests was used. The Biofilm Ring test helps researchers gain information on the formation of biofilms. Understanding the formation of biofilms could help identify a way to break down the biofilm. More problems could arise after the biofilm releases the microorganisms inside. Breaking down the biofilm could result in a greater risk of infection due to the sudden release of microbes. When the biofilm is broken down the microorganisms that would be released would immediately need to be treated with antibiotic, because the organisms are antibiotic resistant and would attach to create a new biofilm. The studies used to write this review did not talk about the potential of what would happen after the break down of the biofilm.

More research into monomicrobial biofilms and their individual antibiotics resistance. In one study, it was shown that the biofilm increased the amount of antibiotic it could withstand when it included *P. aeruginosa*. Studying the single species biofilm could help with the identification of antibiotic targets. Microorganisms that increase antibiotic resistance when in a biofilm should be the main targets for antibiotics. These organisms will contribute to the survival of biofilms. The amount of an organism inside of a biofilm might also contribute to the increased antibiotic resistance. *P.aeruginosa* was shown to have a larger biomass in the biofilm. The factors that contribute to lower cell growth from the other microorganisms could help suppress their growths as biofilms.

Since the strains of *P. aeruginosa* in nature have not been shown to have to ability to hypermutate, more research should be done into what is triggering mutations in biofilms of Cystic fibrosis patients. If something in the treatment of biofilm infected patients is triggering biofilm growth then a new technique needs to be adapted. This knowledge would lead to a greater understanding about mutation in bacterial genomes and could shed some insight into mutations of our own. If an environmental factor plays a role in the development of biofilms, then it could explain the impact that the environment has on our chromosomes in the developing stages.

In patient treatment, using a large amount of antibiotics and increasing antibiotic resistance would

 

potentially harm the patient. The potential harm of the biofilm developing antibiotic resistance in the patient was not addressed in any research done. More research into the treatment of Cystic Fibrosis as a disease should be conducted. Unless there is an advancement in genetics, the only way to help CF patients is the prevention of biofilm growth. The prevention of biofilm growth requires an extensive hygienic routine, combination antibiotic treatments, and DNase spray; this continues on for the patient's life. The only way to prevent lifelong treatments is to fix the genetic disorder, because without the cilia on the patient's lungs the prevention of growth of a bacterial biofilm is difficult.

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