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Biofilm Inhibition: Antibiotics, Genes, and pH

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The effects of biofilms in the health world can be detrimental. Biofilms allow bacteria to resist antibiotics by providing a "slippery" outer layer, thereby inhibiting phagocytosis. One organism in particular that is protected by biofilms is Staphylococcus. This bacterium is antibiotic resistant because of the biofilm. Due to this feature, Staphylococcus is hard to treat and can eventually lead to death. The importance of understanding the bacteria's biofilm will open new possibilities for the creation of biofilm inhibition drugs. The presence of Staphylococcus in society has increased dramatically over the years because many of the current antibiotics no longer inhibit them. In other words, these bacteria have mutated and the rate of infection is high. This is especially true in hospital settings where numerous patients are recovering from an illness or surgery and their immune systems are compromised. Many have observed the growing concern over the inability to stop the rapid influx of staphylococcus cases. In response to this concern numerous studies are being conducted to analyze the bacteria's biofilm in order to understand what allows for inhibition. Although new medications have become available, they are not coming fast enough to keep up with mutating strains.

Introduction

The main concern with biofilm contained bacteria is its ability to resist medications. In hospitals catheters, prosthetics, and various intravenous tubing infection is common. Many surgical procedures provide an open wound allowing easy entry into the body. Some examples include amputation and surgeries requiring a catheter afterwards. Both of which provide a pathway into a "clean" environment. Catheter infections are a common occurrence in hospitals and because the portal of entry is sterile bacteria which do get in are able to thrive. The main concern is biofilm takeover. The biofilm controls the cell as a whole, rendering the host cell to its demands (Dennis). Tissue can become more injured and since the infection is antibiotic resistant the threat of death is high.

Aside from the detrimental aftermath of a biofilm controlled infection, some research has shown specific environments can increase or decrease amounts of biofilm developed. Study two focused on the effect of biofilms in infants. *Staphylococcus* often resulted in low birth weight babies. This study worked specifically with *Staphylococcus epidermis.* Researchers established the controlling environmental factors which included temperature, specific locations, and genes present in bacterial formation (Margaret). Although the subjects of study two were infants and not older adults, the main source of infection was still connected to indwelling devices similar to ones in the first study.

The third study linked both *Staphylococcus aureus* and *Staphylococcus epidermis.* It described these two forms as most common for severe infections. The main purpose of this study was to evaluate effects of pH on bacterial attachment and whether biofilm was affected. The study resulted in positive results and established pH did in fact have a role in biofilm biomass formation (Giuseppe).

Recent Progress

The formation of biofilms is a step-by-step process. Bacteria must attach to the surface of a particular substrate and then multiple bacterial layers are formed. The organisms mature and begin to form a biofilm, and then planktonic cells are released which lead to formation of new biofilms (Dennis). This attachment is an irreversible process. However, researchers identified the main component of the four step process as adhesion. Attachment is crucial for bacteria because without it the infection will remain absent. It has been observed that adhesion mediated by polysaccharide intracellular adhesion (PIA) is an important piece of the overall adhesion process and accumulation of biofilms. It is hypothesized the inhibition of PIA would play a crucial role in the inhibition of biofilm formation and virulence of microbes (Dennis). The study traced synthesis of PIA to a specific gene. This is not the only determining factor of PIA synthesis but it plays a crucial role. A development in the study of biofilm inhibition is a vaccine. Evidence has been reported that antibiotics specifically targeted to PIA or its gene formation reduces the likely hood of biofilm formation. However, devices (i.e. grafts or stents) in the heart, which have bacterial adhesion present, have shown resistance to the previously mentioned antibiotic. This result is believed to occur because the amount of coating surrounding the devices and possible exposure to the bacteria (Dennis). Another problem observed with treating biofilms is the state in which they occur. Bacteria are not always present in the same form and this changing state makes treatment difficult. The growth of bacteria within biofilm thickens the biofilm and makes treatment difficult.

Another important development is recognition of cell "suicide". Biofilm, as previously mentioned, takes control of its host cell. This allows bacteria to obtain food and grow without resistance. However, some waste products of biofilms elicit a small immune response from surrounding tissues. When this occurs biofilm will initiate cell destruction or suicide. However, this destruction may have a reverse effect for biofilm by releasing a segment of DNA which has proven, in some cases, to be crucial in biofilm maintenance (Dennis).

The second article studied environmental factors which may have aided in virulence and production of biofilms. This study focused on low birth weight infants, caused by biofilms, which proved to be at greater risk for sepsis (Margaret). While many studies focused on specific assays against biofilms to determine virulence, researchers of this study tried a different technique and used a variety of chemicals. This technique was chosen to represent diverse environments bacteria are exposed to. In addition to the chemical cocktails infants were kept at a specific temperature. If it was discovered either of these conditions enhanced bacteria's progress or reduced it, proper action could be taken regarding the infant's care (Margaret). The main objective was to determine if invasive coagulase-negative staphylococci (CoNS) or blood culture contaminants differed in production of biofilms. These cases were exposed to different growth conditions and were tested against various skin solutions used in the Neonatal Intensive Care Unit. This provided researchers with data on biofilm production.

Materials used were isolates of blood cultures from infants in the Neonatal Intensive Care Unit located in the Royal Women's Hospital in Australia (Margaret). Polymerase Chain Reaction was used to identify biofilm-specific genes. Samples were broken into two different groups, invasive and contaminants. The study was conducted on biofilms in vitro. Biofilms were subjected to rigorous tests to establish what environmental factors played a role in its production and virulence. It was determined a specific gene, the *ica* gene, was present in biofilm production (Margaret). This same gene was also observed by researchers in the study one (Dennis). In study two involving infants, biofilms lacking the *ica* gene produced weak biofilms. Biofilms which were commensal had the lowest rate of occurrence regarding infection. Whereas blood culture contaminants and invasive isolates, had much higher rates of biofilm production (Margaret). It was discovered biofilms typically produced in hospital environments were resistant to creams and other antibiotic treatments.

In the presence of intravenous solutions biofilm reduction was apparent. Recent progress has led to numerous other studies taking place regarding the *ica* gene. It was hypothesized the isolation and inhibition of this particular gene would decrease cases of *Staph* biofilm production (Margaret).

In the third study the goal was to determine effects of alkaline pH on biofilm production. This is

another representation of environmental factors much like the infant study (Margaret). The alkaline pH effect on biofilm adhesion was the main objective. Teichoic acids (TA) were observed as a key component in production and formation of biofilms (Giuseppe). If the charge of TAs could be altered, then biofilm production was inhibited. Staphylococcal isolates were used in this study. Samples were collected from patients with acquired respiratory or ocular infections. Selection of these particular strains was due to their "well-characterized" biofilm properties. The exact explanation of "well-characterized" refers to the ability to resist antibiotics.

Bacteria were subjected to numerous tests and various levels of pH. Hydrophobicity of the microbial adhesion was measured after all tests had been run. Cultures were then retested in their media with a different pH values. Optical density was then recorded, after pH testing. Bacteria were subjected to other various microscopy scanning and observation of planktonic growth. Effects of pH of planktonic growth were recorded. It was determined pH had an effect on adhesion in the first step of biofilm formation (Giuseppe).

Discussion

Results of all experiments show strides in biofilm understanding. In the end it was determined that inhibiting adhesion or the *ica* gene, was the best way to inhibit biofilm formation. Each study presented their findings in different conjunctions with current research.

In the first study the main focus was on patients in hospital environments. It was determined bacterial presence due to intravenous devices would not be affected by antibiotics because the portal of entry had increased environmental exposure. These open "wounds" provided an environment for bacteria and infection to thrive. (Dennis). There were still unanswered questions about whether antibiotics could keep up with rapidly mutating *Staph* bacteria. Another question to address would be if the environments were altered slightly, the rate of infection would decrease. As in study two, regarding the infants, it was shown temperature had an effect on biofilm formation (Margaret).

In the second study, infants were already in restricted environments. If they were present in a typical hospital setting instead of the Neonatal Intensive Care Unit, the results of the experiment would be different. The answer to this question is important because hospital environments are not consistent around the world. However this study did provide crucial information regarding the *ica* gene effects and other environmental factors (i.e. temperature) on biofilm production (Margaret). These results combined with information gained from the first study would be beneficial in research of biofilm inhibition.

The last study focused on a specific aspect of the environment, pH. It was discovered pH played a crucial role in bacterial adhesion that lead to biofilm formation (Giuseppe). However, pH in the body cannot be easily changed without creating serious health risks. Although pH data is important, the application in patient settings was not discussed. This would be important in understanding how biofilms and bacteria already present in the body could be reduced by a change in pH.

All the studies presented crucial aspects to biofilm development. Each study discussed specific steps of formation and where each study would produce an inhibition. The cases spanned over a variety of patients from severely ill infants to adults with acquired respiratory infections. Due to this broad span many bases of biofilm formation were covered. However, the broad field of study makes results more difficult to combine and determine the best way to inhibit biofilm formation. The studies focused on a crucial aspect of the bacteria which allows them to avoid destruction, mutation.

As previously addressed the genes of bacteria mutate making them less susceptible to antibiotics. When antibiotics become available they must be subjected to rigorous tests before being released for broad use. The problem is antibiotic development takes so long that they often cannot keep up with mutations of biofilms or genes responsible for their adhesion. In the second study results again reiterated the importance of inhibiting the *ica* gene. Since this gene was observed in two completely unrelated studies, the importance of its inhibition cannot be stressed enough. However, the exact way of inhibiting this gene was not addressed. More studies should be conducted regarding isolation and inhibition of this particular gene.

Another important topic to observe was the effects of temperature. In the second and third studies, temperature was used as an environmental

factor. The effects of temperature showed it inhibited the rate of biofilm production and aided in the use of antibiotics, creams, etc. Certain temperatures allowed creams to aid in biofilm inhibition and treatment. In the pH study temperature was used as an aiding agent. It helped altered pH, therefore allowing inhibition of biofilm formation.

All of the studies yielded vital results for further study in biofilm formation. They also addressed the particular process involved in biofilm formation. Then the researchers determined the best way to provide patient care through inhibition and destruction of biofilms. Above all other results researchers established inhibition of virulence in biofilms is crucial. By breaking down the bacteria's virulence, numbers of *Staphylococcus* cases around the world are more likely to decrease.

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