Cystic Fibrosis (CF) is a genetic disease which affects the pancreas, lung, and other organs, which is characterized by the growth of *Pseudomonas Aeruginosa* (PA) biofilms in the lungs, causing a vast number of symptoms that affect lung ability and efficiency [1]. There are many medications used to help the symptoms of this disease, but there are no drugs used to kill biofilms, due to biofilms having the ability to remain resistant to most drugs [20]. The biofilm and glue-like mucus, which is secreted by the bacteria, cause antibiotics and other drugs to be rendered useless against killing the bacteria [2]. Killing this barrier to the lungs, would potentially prevent respiratory failure and the progression of CF, better than the current treatments. Recently, a development was made in that *Benzoyl Peroxide* (BP) and *Salicylic Acid* (SA) was able to inhibit biofilm formation and to reduce biomass in *Bacterial Vaginosis* (BV) [3] and *Escherichia Coli* (EC) [9].

We look for information of the biofilm barrier to kill mucus and increase the effectiveness of current CF lung medication. As seen in the BV development, we hypothesize that if we add the drug to a carrier molecule, the drug would attack and inhibit the biofilm barrier. In doing so, we hope to prevent further degradation of lung function and to inhibit the effect of CF. We plan to identify a pairing of a carrier molecule and drug to inhibit biofilm growth and to continue to degrade the mucus and biofilm (Aim 1). We will identify if the use of a carrier molecule delivery system increases the efficiency of drug delivery by measuring the effect of the drug on pathogenic biofilms associated with CF. (Aim 2).

<u>Aim 1</u>: Identify a combination of mucus permeable molecule and suitable drug for degradation of biofilm.

A. Compare the benefits and the disadvantages associated with the mucus permeable molecule in the lungs.
B. Compare the benefits and the disadvantages associated with the drug in the body.

<u>Aim 2:</u> Identify if the use of a carrier molecule delivery system increases the efficiency of drug delivery by measuring the effect of the drug on pathogenic biofilms associated with CF.

A. To determine if the polymeric nanoparticle delivery system allows for a higher percentage of the drug to be delivered to the targeted area.
B. To determine the drugs efficiency against pathogenic biofilms and the progression of CF, by simulation.

<u>Background</u>:

Cystic Fibrosis: *Cystic Fibrosis* (CF) is a genetic disease which is expressed when both parents have at least one copy of the gene. The *Cystic Fibrosis Transmembrane Conductance Regulator* (CFTR) protein becomes defective when the CFTR has a mutation present [12]. The protein is unable to move chloride to the surface of the cell, which causes the production of mucus, due to the lack of water present at the cell

surface. This chronic illness is characterized by the production of mucus, which causes a buildup of biofilm in the lungs and pancreas, causing lung infections, digestion problems, inflammation, and respiratory failure [1].

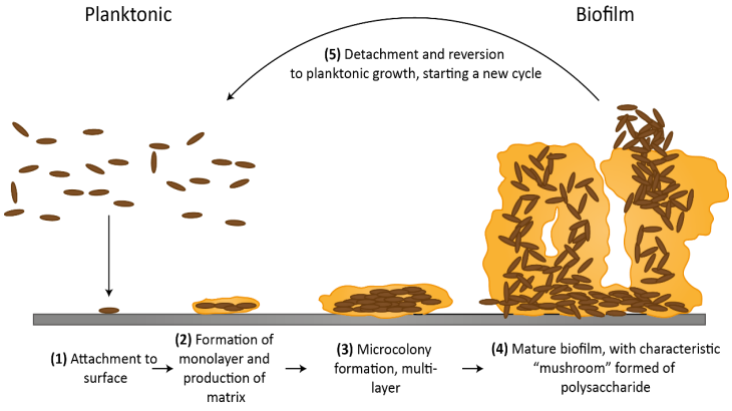

Figure 1: The Biofilm adhere to the surface, where the bacteria continues to proliferate, causing an irreversible , 3D figure of bacteria, known as a "Biofilm." Source [6].

Biofilm: When microorganisms proliferate and continue to build up, they create a biofilm, usually consisting of bacteria. Since the bacteria accumulate in significant numbers, they are in close proximity, which allows the bacteria to support each other. This allows the bacteria to exchange substrates and share metabolic products, which allows the bacteria to use their structure to protect the integrity of the biofilm, which are also usually made up of different bacteria [14]. Biofilm is protected from the immune system, antimicrobials, and some drugs [16]. The bacteria comes in contact with the surface (**Figure 1**), where it begins to form a monolayer which eventually produces an *Extracellular Matrix* (ECM). The ECM is also known as mucus, which is a form of protection for the biofilm. Eventually, the bacteria continues to grow to a 3D structure at a very significant rate, which causes the attachment to become irreversible [6]. Biofilms can acquire resistance to antibiotics by changing cellular structure and producing enzymes that render the drugs ineffective [5].

Biofilm in Cystic Fibrosis: Chronic *Pseudomonas Aeruginosa* (PA) is a bacterium which creates biofilm in the lungs of patients with CF. PA changes from an acute pathogen, to a pathogen which is adapted to CF, which is due to the polysaccharide alginate overproduction, which causes the biofilm to form and produce a hydrogel, known as mucus [15]. The biofilm is antibiotic resistant and phagocytosis resistant, which causes chronic inflammation, lung damage, and lung infections [6]. Once PA forms a biofilm, the infection becomes irreversible and is virtually impossible to destroy. Over 80% of patients with CF are overcome by respiratory failure, which is due to the biofilm and inflammation of the airways [7]

BV and E. Coli Breakthrough: Recently, a study was performed in which *Benzoyl Peroxide (BP)* and *Salicylic Acid* (SA) was used to attack the biofilm formed by *Gardnerella Vaginalis* (GV). An experiment was performed in which concentrations of 250µg/mL and 125µg/mL of *Benzoyl Peroxide* and *Salicylic Acid* were used in assays against the biofilm to see if the biofilm could be killed or at least broken down. The results showed the in concentrations greater than 250µg/mL, BP and SA were able to inhibit the growth of GV, and that further studies will be done to see the effects of BP and SA on other bacterial biofilms [8]. *Salicylic Acid* has also been used in a study with *Escherichia Coli*, where it was again proven that SA reduces bacterial adhesion, reduces biomass, and affects the development of the biofilms structure [9].

Experimental Design:

Aim 1: Identify a combination of mucus permeable molecule and suitable drug for degradation of biofilm. It is known that there are many different biomaterials available to transport drugs through various body systems, allowing for the drug to reach the targeted destination. Finding the best biomaterial for this drug delivery application, and pairing it with a drug to help CF, may allow for a more suitable treatment.

Aim 1A: Compare the benefits and the disadvantages associated with the mucus permeable molecule in the lungs. First, we need to identify different biomaterials that are suitable options for drug delivery. Each identified biomaterial will be tested on multiple criteria aspects, where they will be ranked from most effective (higher number) to least effective (lower number). The biomaterial with the highest number will be named the most effective biomaterial for this experiment. The three different biomaterials that will be tested are: *Polymeric Nanoparticles, Micelles, and Dendrimers* [13]. The list of test criteria includes, but is not limited to:

The transportation rate to the lungs: the different biomaterials will be dyed using *Coumarin 6* (fluorescent dye), which will allow us to image the particles at various times, allowing for the transportation rate to be calculated. The biomaterials will be injected into mice, where the microparticles will be imaged and quantified frequently, allowing for the fastest biomaterial to enter the bloodstream and diffuse into the lungs [10].

The ability to penetrate the mucus: the different biomaterials will again be dyed using *Coumarin 6*, but they will be injected into a collected mucus gel. The biomaterials should be tested in two different collected mucus: *Pseudomonas Aeruginosa* and *Gardnerella Vaginalis*, allowing us to see if there are any differences in mucus penetration. The fluorescent biomaterials will be imaged frequently to see the process of penetration into the mucus. Alternatively, we can use confocal microscopy (this microscopy allows us to produce high quality images) to do this trial as well [21]. This process will also be timed, allowing us to see which biomaterial can penetrate the mucus in the least amount of time, but also shows us which biomaterial penetrate the mucus most efficiently [10].

The duration of Bioadhesivity: the different biomaterials will be filled with fluorescent *Bovine Serum Albumin* (BSA), and then they will be placed on the *Pseudomonas Aeruginosa* biofilm, where the bioadhesive will be measured. There will be at least three trials for each of the different biomaterials, allowing us to see if they can remain adhered after a short duration of time (.5-1 hour), a medium duration (2-3 hours), and a long duration (6+ hours). After the time duration is up, we will attempt to wash the biomaterial off the surface of the biofilm, to allow us to see which biomaterials remained adhered and which did not [10].

Aim 1B: Compare the benefits and the disadvantages associated with the drug in the body. We are looking for the best drug to combat biofilms, which will prevent mucus, therefore helping patients with CF. It is important we know how effective or ineffective this drug will be against biofilms. We will use many tests to see how this drug affects the user, which includes, but is not limited to:

The ability to attack the biofilm, but not negatively affect the body: The purpose of this drug is to attack the biofilm and prevent further growth of it. The drug needs to be able to make it to the lungs, where it then needs to be able to attack biofilms such as *Pseudomonas Aeruginosa* and *Gardnerella Vaginalis*. It is important that the drug is able to attack the biofilm, but minimize the damage to the rest of the lungs and the body. We can test if the drug is effective against biofilms by using *Coumarin 6*, allowing us to continuously image this process, which allows us to visually see how this drug affects the biofilm, while at the same time, watching to see how the drug affects the lungs and the rest of the body. This stage of testing may take a while due to testing many different drugs and comparing their results, but we want to focus on testing with BP and SA against *Pseudomonas Aeruginosa* due to the results of the BV experiments.

Aim 2: Identify if the use of a carrier molecule delivery system increases the efficiency of drug delivery by measuring the effect of the drug on pathogenic biofilms associated with CF. We believe that a carrier molecule delivery system will increase the efficiency of the drug against the biofilms in CF affected lungs. We hypothesize that the drug will be delivered more efficiently, allowing for a larger percentage of the drug to be delivered to the targeted area.

Aim 2A: To determine if the carrier molecule delivery system allows for a higher percentage of the drug to be delivered to the targeted area. We believe that using a carrier molecule delivery system will be more effective against biofilms than antibiotics are. The biofilm can become resistant to the antibiotics, and antibiotics offer more side effects due to a larger amount of the drug accidently being delivered to the non-targeted area.

Percent of the drug that is delivered to the targeted area: To test how much of the drug is being delivered to the targeted area by using *Coumarin 6* to dye the drug/carrier, allowing us to image the body continuously. This will allow us to track where the drug is ending up, allowing us to see if a significant amount of the drug is being delivered to the targeted area.

Aim 2B: To determine the drugs efficiency against pathogenic biofilms and the progression of CF, by simulation. The goal of this experiment is to delay the progression of CF, allowing for the patient to live a longer life with fewer symptoms of CF. In order to determine if the drugs are efficient, we need to test multiple aspects, which includes, but is not limited to:

The Ability to Damage the Biofilm and Inhibit Growth: The purpose of this drug is to destroy a majority of the biofilm and prevent further growth of it. This will be tested by

comparing the size of the biofilm, to see if the biomass has decreased by the end of this trial. We can also test if the drug is effective against biofilms by using *Coumarin 6*, allowing us to continuously image this process, to allow us to visualize how this drug affects the biofilm. Specifically, we want to test BP and SA against *Pseudomonas Aeruginosa* due to the results of the BV experiments.

The Progression of CF: To measure the progression of CF, we can take a look at how the biofilm has been affected by the drug, it will get smaller due to bacterial necrosis, or it will get larger due to bacterial proliferation. The patients' health will begin to worsen over time if the biofilm is not dying off. This experiment will take a long period of time, because we have to look at the long-term effects as well as the short term effects. We specifically want to test BP and SA against *Pseudomonas Aeruginosa* due to the results of the BV experiments.

Significance: We are fortunate to be living in an era where technological advances are allowing humans to live in a better state of health, for much longer. Unfortunately, CF still has no cure, causing thousands of people to suffer each year [17]. *Cystic Fibrosis* is typically diagnosed as a child, allowing the averaging a life expectancy of 37.5 years [11], with over 30,000 people affected in the United States alone [1]. The current drugs are inhalation drugs to allow for higher pulmonary concentrations, but there are limitations due to the bronchial obstructions [18]. It has been tested and shown that nanoparticle drug delivery systems are better at eradicating the biofilm layer, showing that particle drug delivery systems are the future of CF [19]. If we find a drug that can attack and inhibit the biofilm, we may be able to help those with CF, allowing them to live a longer life, in less pain. *Benzoyl Peroxide* and *Salicylic Acid* have had promising results in other trials, making those drugs more viable contestants to help battle the biofilm in CF.

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