

# Biofilm Reduction In Simulated Cystic Fibrosis Lungs Using Novel Methods

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## Abstract

Cystic fibrosis is caused by an autosomal recessive disorder that leads to inflammation of the lungs and excess mucus production, eventually leading to progressive lung injury and deterioration. This disease is further complicated by chronic mucoid infections from *Pseudomonas aeruginosa*, a commonly found bacteria that is resistant to most antibiotics due to production of a protective barrier called a biofilm. It is the aim of this research to investigate novel methods of biofilm reduction using innovative techniques. Varying concentrations of the stilbene resveratrol, and the chelation therapy drug Ethylenediaminetetraacetic acid (EDTA), which have been shown to degrade biofilms. Testing will be composed of two phases of testing: first, establishing biofilm formation from the strain of *Pseudomonas aeruginosa* obtained and the most effective concentrations of resveratrol and EDTA in biofilm reduction. The second phase of testing will include obtaining a sterile 3D printed anatomical replicate of a fetal cystic fibrosis lung and inoculating it with the biofilm producing strain of *Pseudomonas aeruginosa*, then incubating the lung overnight with the most effective drug concentrations from phase 1. It is the expectation of this study to observe significant biofilm reduction in a complex, anatomical replicate utilizing proven additives in an innovative way.

## Introduction

Cystic Fibrosis is a hereditary disease that causes lung inflammation and an overproduction of mucus, which creates a suitable environment for a bacterial infection. *Pseudomonas aeruginosa* is the most commonly isolated bacteria in Cystic Fibrosis patients, and is often difficult to treat due in part to its biofilm production. This research seeks to implement new method of biofilm reduction within a Simulated Anatomical Matrix (SAM) that represents a Cystic Fibrosis lung.

## Cystic Fibrosis & *Pseudomonas aeruginosa*

An autosomal recessive disorder that leads to inflammation and the overproduction of mucus in the lungs. The excess mucus can be treated with mucolytics, however when a mucoid bacterial infection occurs it can be more difficult to get rid of the excess mucus. *Pseudomonas aeruginosa* is the most common pathogenic organism cultured from Cystic Fibrosis patients, and in this study the strain of bacteria is American Type Culture Collection number 27853.

## EDTA & Resveratrol

EDTA is a chemical compound that is often used in chelation therapy in heavy metal poisoning. EDTA has also shown to possess antimicrobial properties such as breaking down mature biofilms. Resveratrol is a plant derivative with antimicrobial properties that inhibits quorum sensing in biofilm forming bacteria.



Image 1- This image depicts a dissected cystic fibrosis lung, showing the accumulation of mucus throughout the airways. Source: <https://clinicalgate.com/endocrine-system-and-nutritional-and-metabolic-diseases/>



Image 2- The *Pseudomonas aeruginosa* was streaked onto a Sheep Blood Agar plate to obtain isolated colonies. Shown here are those isolated colonies, which are greyish in color.

## RESULTS

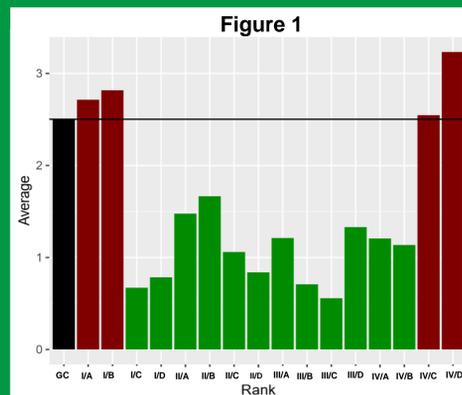


Figure 1: Biofilm Reduction in 96 micro-well plates. This graph represents overall biofilm production when incubated with varying concentrations of drug combinations. Roman numerals represent EDTA (I-2%, II-1%, III-0.5%, IV-0.25%) and letters represents resveratrol (A-2%, B-1%, C-0.5%, D-0.25%).

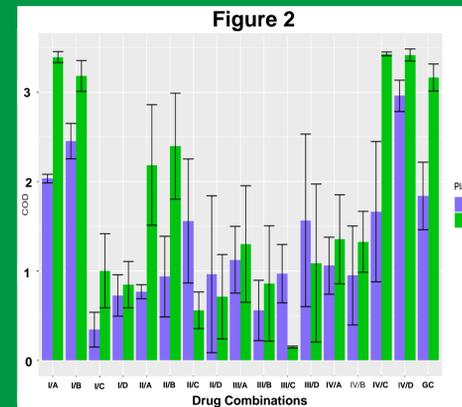


Figure 2: Comparison of Drug Combinations in the two plates. This graph shows the overall biofilm production when incubated in the presence of the two drugs. Error bars were placed to show variation between the two groups. Roman numerals represent EDTA (I-2%, II-1%, III-0.5%, IV-0.25%) and letters represents resveratrol (A-2%, B-1%, C-0.5%, D-0.25%).

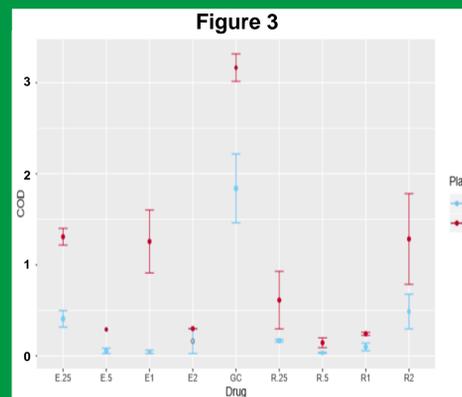


Figure 3: Plate Comparison by Individual Drug Concentrations. This graph shows the average of each drug concentration with error bars to indicate the amount of variation between the groups, including the removal of outliers.

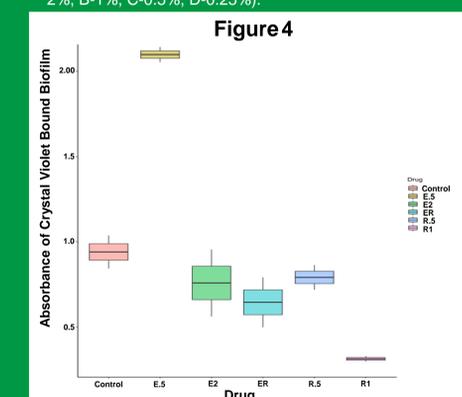


Figure 4: SAM Lung Biofilm Reduction. This graph represents average biofilm reduction in the SAM lungs up to +/- 2 SD from the mean. The "E" represents EDTA, and the "R" represents resveratrol, each at their concentration at the time of testing.

## Methods

### Drug Concentrations

For both phases, the drugs were made into highly concentrated stock solutions by combining EDTA with distilled water and resveratrol with reagent grade ethanol. In the first phase, each drug was diluted with distilled water to concentrations of 2%, 1%, 0.5% and 0.25%. In the second phase, 1%, 0.5%, of each drug was used. Due to prior research showing safe concentrations of EDTA and resveratrol, these were our target concentrations.

### Plate Preparation

*Pseudomonas aeruginosa* was inoculated into trypticase soy broth (TSB) at 0.12 Optical Density (OD), which was confirmed using nephelometry. This solution was placed into a 96-micro well plate and diluted down to research standard of 0.06 OD with the drugs solutions. This solution was combined with the drugs and incubated overnight.

### SAM Preparation

The SAM was printed by the Department of Radiologic Sciences, but to sterilize the prints, an innovative method was utilized. The prints were immersed in reagent alcohol for 10 minutes, left under UV lights for 30 minutes, then microwaved in sterile reagent microwave for 30 seconds. The SAM was then inoculated with *Pseudomonas aeruginosa* placed in trypticase soy broth (TSB) at 0.12 Optical Density (OD), and diluted to 0.06 with drug solutions.

### Wash and Stain

After incubation, the 96-microwell plates and lungs were then washed 4 times with sterilized PBS and stained with crystal violet for 10 minutes. After staining, the plates and lungs were again washed with PBS to remove the unbound crystal violet until the fluid was clear.

### Absorbance Reading

Reagent grade ethanol was used to resuspend the stained biofilm. This solution was then transferred to a sterile 96-microwell plate, and read an optical density at absorbance of 595 nm. Values obtained from these readings were then averaged according to drug concentrations ran in combination and drug concentrations ran by itself. The values obtained from the SAM models were compared to a single growth control established before testing drug concentrations, and the plates were run with a positive and negative growth control per plate.

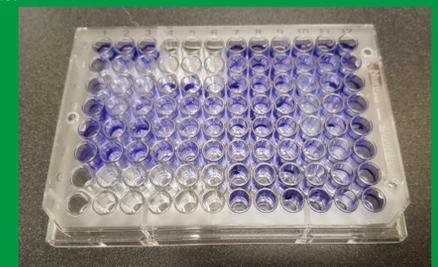


Image 3- This image shows the 96-microwell plate used in this study, with the stained biofilm attached to the sides of the well.

## Discussion

- The most effective concentrations of EDTA and resveratrol by themselves was between 0.5% and 1% in the microwell plates. While effective in the SAM, these results were not statistically significant according to R studio programming.
- The most effective concentrations of the combinations in the plates were 0.5% EDTA & 0.5% Resveratrol. In the SAM however, we utilized 1% for the combination due to an increased total volume, which was shown to be not statistically significant according to R studio programming.
- The combination with the highest concentrations of drugs in the microwell plates showed an antagonistic effect and didn't show any biofilm reduction.
- Resveratrol at 1% was the best at reducing biofilm in the SAM lungs, and was also shown to be statistically significant using R studio programming.

## Limitations

- We didn't have enough time to run more SAM replicates for more data.
- The 3-D printing wasn't performed in a sterile environment, and so each print had to be thoroughly sterilized prior to testing.



Image 4- This image is a SAM of a Cystic Fibrosis lung used in a prior experiment.