# Repurposing Microbes as Living Biologic for Antibiotic Resistance

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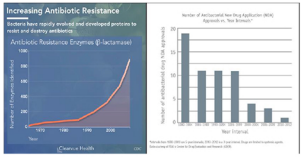
## Introduction/Background

Antibiotic resistant diseases are a huge problem across the world as traditional antibiotics are becoming ineffective on these diseases.

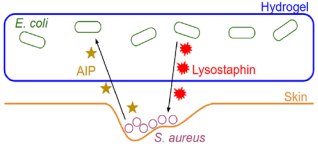



One antibiotic resistant strain common worldwide is methicillin-resistant *Staphylococcus aureus* (MRSA).

According to the CDC, there were nearly 120,000 cases of bloodstream *S. aureus* infections in the United States in 2017, of which more than 20,000 resulted in the death of the patient (CDC, 2019 March 5).

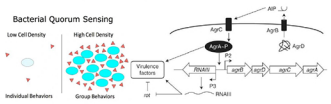
Our goal is to create a wound dressing that will fight against MRSA infections by:

- Using *Escherichia coli (E. coli)* that has been genetically engineered to combat MRSA.
- Fabricating a hydrogel-based wound dressing that can be easily applied to promote wound healing.
- Designing a model to predict the efficiency of the genetic circuit.




Using this strategy, it can be possible to apply this bacteria-killing technique to many other types of antibiotic resistant diseases, not just fighting MRSA.

## Genetic Engineering




**Tapping Into Inherent Bacterial Communication System:**

- Quorum sensing occurs when the cells have a population over a specific threshold which synchronize group behavior.
- Using *S. aureus's* natural communication system and splitting it between two plasmids, we can use it to sense when MRSA is present and genetically engineer a release of a kill mechanism.

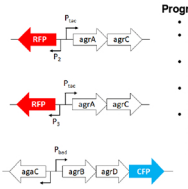
**Design of Genetic Switch**

Plasmids that sense different levels of AIP

- *agrAagrC* – P2
- *agrAagrC* – P3
- The two-sensing plasmids will allow us to determine sensitivity of promoters and work with the one that is the most selective to AIP.
- RFP will fluoresce to prove circuit works, it is a substitute for lysostaphin, which would kill MRSA.

Plasmid that produces AIP

- *agrBagrD*
- AIP is expensive to buy and if synthesized by *E. coli*, we could have the ability to not only produce AIP but also perform several co-culturing experiments.




**Progress to Date**

- Three plasmids designed and synthesized from IDT.
- *agrBagrD* was synthesized and transformed into *E.coli* cells, with a confirmed sequence.
- *agrAagrC* – P2 had several sequencing mistakes that we were unable to fix.
- *agrAagrC* – P3 had 2 base pair mutations. In process of replacing them and transforming into *E.coli* cells.
- Unable to transform *agrAagrC* – P3 into regular chemically competent cells; in the process of transforming into laq Iq *E.coli* cells which would allow for the repression of toxic proteins.

## Hydrogel

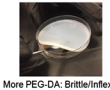
Hydrogel Precursor Solution

- Distilled water 33.9% v/v
- Poly(ethylene glycol) diacrylate (PEG-DA) 8.9% v/v
- 2-Hydroxy-2-methylpropiophenone (HOMPP) 0.3% v/v
- Sodium alginate 7.85 g/L

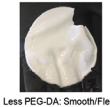
Exposed precursor solution to a broad-spectrum ultraviolet (UV) light, causing the molecules to crosslink.

The mechanical properties were optimized.

- Completed 19 iterations over the semester, varying UV exposure times and the composition of the precursor solution.



More PEG-DA: Brittle/Inflexible



Less PEG-DA: Smooth/Flexible

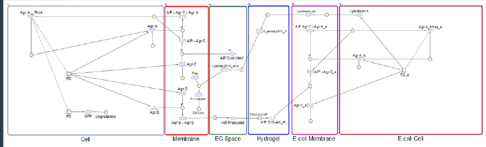
To be sure that the UV light would not kill the cells inside the hydrogel, we exposed *E. coli* cells to UV light for various periods of time and plated the resulting cells.

| Dilution of cells | 1 minute of UV exposure | 2 minutes of UV exposure | 5 minutes of UV exposure | 10 minutes of UV exposure | 15 minutes of UV exposure |
|---|---|---|---|---|---|
| 10^-6 | Growth | No Growth | No Growth | No Growth | No Growth |
| 10^-7 | No Growth | No Growth | No Growth | No Growth | No Growth |
| 10^-8 | No Growth | No Growth | No Growth | No Growth | No Growth |

To ensure that no *E. coli* could escape the hydrogel, and possibly get on the wound, *E. coli* immobilization tests were performed.

Control Plate | Results: No *E. coli* growth under hydrogels

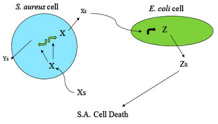
## Modeling



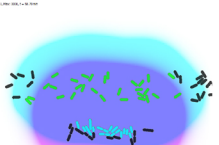

- Expanded ODE system translated from gene regulatory networks
- Multi-Compartment Analysis

- Based on Mass Action Kinetics
- Stochastic system that tracks molecule counts over long periods of time

Production (Hill Function)
$\Phi \rightarrow X$ w/ rate $f_1(X) + f_2(X_s)$
$\Phi \rightarrow Y$ w/ rate $g_1(X)$
$\Phi \rightarrow Z$ w/ rate $h_1$
Diffusion (Fick's Law)
$J_{xs} \rightarrow J_1(X)$
$J_{ys} \rightarrow J_2(Y)$
$J_{zs} \rightarrow J_3(Z)$
Degradation (Constant)
$X \rightarrow \Phi$ w/ rate $\gamma_x$
$Y \rightarrow \Phi$ w/ rate $\gamma_y$
$Z \rightarrow \Phi$ w/ rate $\gamma_z$
Death Rate (Hill Function)
Death = $q_1(Z_s)$




- Simple 3 Signal Stochastic Model
- Understand how system works intuitively
- Modeled in GRO to see real time evolution of cells



## Conclusions

Genetic Engineering

- Proof of concept circuits could be created using our designed plasmids and synthetic biology techniques.

Hydrogel

- Hydrogel is flexible and shows promising results for immobilizing *E. coli*.
- Tests show *E. coli* cells able to survive under UV exposure for up to 1 minute.

Modeling

- Simple stochastic models can give rise to intuitive understanding of the underlying system.

## Future Directions

Genetic Engineering

- Complete construction of proof of concept P2 and P3 plasmids.
- Sequence confirm P2 and P3 plasmids.
- Move confirmed parts to *Staphylococcus carnosus (S. carnosus)*.
- Begin co-culturing experiments with and without hydrogel.

Hydrogel

- Continue with *E. coli* immobilization tests.
- Perform AIP and lysostaphin diffusion tests.
- Consider ways to secure hydrogel onto wound.
- Consider storage and transportation of hydrogel.

Modeling

- Continue model to represent entire system.
- Gather quantitative experimental data from hydrogel and genetic engineering experiments.
- Parameter fit model to experimental data.

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