Using a highly expressed cyclic-di-GMP aptamer to affect biofilm formation


**PROBLEM**

- Biofilms are bacterial growths that form on surfaces when bacteria experience stressful conditions.
- The bacteria secrete an extracellular matrix that promotes attachment, excludes immune cells, and increases antibiotic resistance.
- These biofilms form in wounds, lungs of cystic fibrosis patients, chronic infections, and inserted medical devices.
- The CDC estimates that 65% of all infections in developed nations are the result of biofilms.
- They are the leading cause of Healthcare Associated Infections.

**BACKGROUND**

- Cyclic-di-GMP is a second messenger universal in biofilm signaling.
- Increased [c-di-GMP] leads to increased biofilm formation.
- Decreased [c-di-GMP] leads to increased motility and flagella expression.
- *B. bacteriovorus* has a massively expressed regulatory RNA (merRNA) that contains a c-di-GMP aptamer.
- This is hypothesized to sequester c-di-GMP and therefore promote motility.

**EXPERIMENTAL DESIGN**

The merRNA transcript will bind to and sequester c-di-GMP. Lower intracellular c-di-GMP concentrations inhibit biofilm formation.

**RESULTS**

**Initial Results**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Biofilm formation (30°C)</th>
<th>Biofilm formation (25°C)</th>
<th>Biofilm formation (37°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>0.5</td>
<td>0.2</td>
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</tbody>
</table>

**Biofilm Formation**

- Grow bacteria statically in a 96-well plate
- Remove planktonic bacteria and rinse. Only cells stuck in a biofilm remain behind.
- Stain with crystal violet and rinse
- Dissolve crystal violet in acetone/ethanol
- Record A550

Contrary to expectations, cells expressing the merRNA exhibited increased biofilm formation. This was observed most consistently at lower temperatures, which tend to incur a metabolic burden and favor biofilm states.

**Human Practices**

**Phage Encapsulation in Silk Bandages**

"Tufts Synthetic Biology envisions a biomedical product consisting of a silk film embedded with a lyophilized cocktail of bacteriophage-targeting pathogens responsible for chronic wound infection."

**Bacteriophage**

- Growing antibiotic resistance perhaps most critical concern of modern medicine
- Deaths caused by antibiotic resistance top $23,000 per year in the U.S. alone
- U.S. economic cost due to antibiotic resistant infections upwards of $50 Billion per year
- Bacteriophages are viruses which target specific bacterial strains
- Approved for food, agricultural, and environmental use by FDA, USDA, and CDC
- Utilised therapeutically against bacterial infections in Republic of Georgia since 1930’s
- Face negative stigma and insufficient, credible research in Western medicine

**Silk – Promising Platform for Bacteriophage Delivery & Wound Healing**

- Thermo-stabilizes bacteriophages, Confers moisture resistance
- Increases distributive ability for elimination of expensive and electricity-based storage for developing countries
- Biocompatible, Promotes wound healing, Directed Dissolution and compound release

**Bandage**

- Encapsulate bacteriophage cocktail within silk for treatment of Methicillin-resistant Staphylococcus aureus (MRSA)
- Utilized against antibiotic resistant infections aids in more rapid approval and use by the medical industry
- Potential for treatment of combat wounds, household wounds, burn victims, etc.

**REFERENCES**


**ACKNOWLEDGMENTS**

- Biology of Phage: Tufts Synthetic Biology
- Phage History and Current Use: Anna Kuchment
- Current Research: Natural Phage - Dr. Andrew Camilli
- Current Research: Engineered Phage – Mark Mimee
- Distributive Action: Christopher Ghadban
- Bandage: – Anna Kuchment

**FUTURE WORK**

- Improved delivery techniques
- In vivo studies
- Comparative studies with other biofilm inhibitors

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