Biosensors for Antibacterial Medical Applications

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Abstract
Healthcare associated infections are significant concern in medicine and as antibiotics are becoming less effective we need new alternative methods to treat them. We are testing various materials that can be used to prevent infections in healthcare settings and in wound treatments. These materials include bioactive glass and modified hydrogel materials. From these studies we’ve learned these are effective in killing bacteria and preventing infections. The goal of this project is to determine the mechanism at which these materials kill bacteria. Our approach is to construct biosensor strains that can indicate bacterial stress response to antimicrobial agents. We have used polymerase chain reaction (PCR) to amplify promoters from known bacterial stress response genes. These include recA, lasR, and grpE. We are cloning these promoters in front of a promoter less lacZ gene to serve as an indicator of bacterial stress in response to antibacterial materials.

Background
Need for antimicrobial materials to combat hospital acquired infections and reduce antibiotic resistance. Such infections result in systemic disease and may require prolonged treatment with antibiotic which could lead to septic shock. Therefore, it is desirable to prevent infections by inhibiting bacteria from establishing an infection in the first place. One approach we are testing is the use of biosensors. Biosensors will indicate the mode of action- recA (DNA damage), grpE (metabolic stress), lasR (general stress). Advantages of biosensors include: non-toxic, rapid reaction response, biodegradable, minimal scarring, easily handled, and stimulates the body to start the healing process.

Experimental Procedures
Testing Antimicrobial Activity
We are interested in the antimicrobial activities of hydrogels doped with antibacterial compounds such as Mxene and antibiotics. Bacteria to be tested were spread on trypticase soy agar plates to create a lawn. Wells were cut into the agar plate and filled with hydrogel and incubated at 37 degrees C for 24 hours.

Polymerase Chain Reaction
We’ve designed PCR primers for the promoter for the three genes associated with stress whose expression is linked to interaction with various antibiotics (recA, and grpE from E. coli and lasIR from P. aeruginosa). The PCR product is purified using a Gel/PCR extraction kit before being used for cloning.

Plasmid Preparation
The lacZ plasmid is cut with restriction enzymes BamHI and HindIII to facilitate cloning the PCR product into the plasmid. Using two different enzymes prevents the plasmid from recombining with itself. The cut plasmid DNA is purified using a Gel/PCR extraction kit before being used for cloning.

Sub Cloning Genes into Plasmid Vector
To the left is a diagram of how we clone our promoter into our lac gene plasmid using homologous recombination – a technique called Gibson Assembly. If our hypothesis is correct, promoter will get turned on by exposing the bacteria to antibiotics and will turn blue in the presence of X-gal. Notice how in the plasmids, the blue and red separate, making room for our PCR product to be recombined into the vector.

Progress Report
We tested various combinations of hydrogel with MXene and heparin and ampicillin against Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus epidermidis. Only the combination with ampicillin was effective.

For PCR we used the appropriate bacterial DNA as our template and obtained positive PCR products of the expected sizes. Therefore, we can confirm that the primers used were correct. We now have PCR products containing the promoters to clone next to the lacZ indicator gene.

The gel represents the pLac plasmid that will be used to clone the PCR amplified DNA from the step prior. The plasmid was cut with restriction enzymes EcoRI and XbaI to facilitate cloning the PCR product into the plasmid. The DNA was purified using a gel/pcr clean-up kit after running the gel.