Metallic Nanoparticle Effects on Microbial Organisms

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Summary of objectives and significance of proposed research.

The benefits of ionic copper, silver (Cu\(^{2+} \approx 0.3\) mg/L, and Ag\(^+ \approx 0.02\) mg/L) and CuO usage in the treatment of bacterial outbreaks are effective and cost efficient solutions to the ongoing struggle of increased microbial resistance to other forms of antibacterial products such as medications and disinfectants. Few studies have been conducted on the modality of metallic nanoparticle disruption on bacterial cell systems, thus we propose the construction of biosensor strains of *Escherichia coli* with the capabilities of responding to various types of cell damage.

Biosensor strains of *E. coli* will be constructed using a synthetic biology approach. This approach takes advantage of a promoterless bioluminescent *lux* operon by fusing it with three variations of cellular damage sensing promoters: *recA* (DNA damage), *grpE* (heat-shock - general metabolic stress), and *lasRI* (quorum sensing). This strategy has been successfully used by Eltzov *et al* (reference) to identify the mode of action of antibiotics based on their pattern of inducing luminescence. Therefore, by exposing the biosensor strains of *E. coli* to metallic nanoparticles, the mode of damage can be qualified through observation of bioluminescence. Coupled with the use of SC-ICP-MS and SP-ICP-MS, precise nanoparticle amounts needed to cause bacterial cell death can be determined for the eventual medical application of metallic nanoparticles. Hypothetically, the application of these nanoparticles will behave similarly to antibiotics concerning the caused bacterial cell damage, and in the unlikelihood they do not have a similar behavior, the nanoparticles will still cause a particular pattern of luminescence via activation of the promoter and subsequent *lux* operon.

Summary of project materials and suitability of methods to be employed.

Experiments will be conducted to construct the biosensor strains of bacteria. To construct these strains, primers have been designed to PCR amplify each of the promoters. Polymerase chain reaction (PCR) will be used to amplify each promoter sequence. The amplified promoter sequences will be cloned into plasmid DNA vectors designed for cloning PCR products for further manipulation. The PCR primers were designed to incorporate specific restriction endonuclease cleavage sites upstream and downstream of the promoter to facilitate further cloning steps. The promoters will be fused to the promoterless *lux* operon by splicing open the vector containing the *lux* operon through usage of the appropriate restriction enzymes, and ligating the promoter sequence into the vector. Following the assembly of the biosensor strain, PCR will be performed to amplify the DNA to visually determine the success of the strain construction after running the DNA through gel electrophoresis.

Following the successful completion of biosensor strain construction we will begin experimentation of metallic nanoparticle effects on each biosensor strain. The biosensor strain will be exposed to varying concentrations of both silver and copper nanoparticles. As we are working with three different promoters, we will be able to determine what effect is created by the application of metal nanoparticles whether it damages the cells DNA, causes heat shock, induces quorum sensing, or a combination of all three. The biosensor system works best when working with a sublethal dose of the antibacterial agent. Therefore, we will be simultaneously measuring cell survival to determine the relative amount of nanoparticles necessary to kill the bacteria. To
more precisely determine the correlation between nanoparticle concentration and cell death, we will work with the laboratory of Dr. Honglan Shi on the application of single-particle inductively coupled plasma mass spectrometry (SP-ICP-MS) and single-cell ICP-MS (SC-ICP-MS) to measure the amount of nanoparticles per bacterial cell.

**Timeline for student engagement**

The goal of this project is to have it completed within one academic year. Beginning in the Fall of 2021, the first goal of the project is to successfully amplify the needed promoters and subsequently, successfully construct the biosensor strains. This process will be achieved through the usage of PCR and eventual cloning of the amplified promoters into a T-vector. The promoters will be amplified using DNA from the appropriate bacteria as template (*Pseudomonas aeruginosa* for *lasRI* and *E. coli* for *recA* and *grpE*). Following this cloning process, the biosensor promoter fragment will be cut with restriction enzymes and cloned into the *lux* plasmid. Successful cloning of the promoters to the *lux* operon will be verified by gel electrophoresis and DNA sequencing. The biosensors will be tested against antibiotics known to induce luminescence as done by Eltzov et al (reference).

Following the successful construction of the biosensor strain in the Fall of 2021, the project will then move into application of metal nanoparticles in the Spring of 2022. During this time, the student researcher will work to determine the effects of silver and copper nanoparticles on the biosensor strains. They will identify the needed concentrations of nanoparticles to affect the bacteria’s structural integrity as it would apply for use for a medical or disinfectant purpose. This will involve the use of cell viability assays and live-dead staining of the bacterial cells during exposure to the nanoparticles.

**Student researcher qualifications**

Antimicrobial Activities of Bioactive Glass With and Without Silver
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Dr. David Westenberg

My research team performed experimentations on *Staphylococcus saprophyticus*, a gram-positive bacteria, as well as *Staphylococcus epidermidis* and *Staphylococcus aureus* which are gram-positive bacteria and bioactive glass. The goal was to determine their susceptibility to silver at varying pH via the application of silver infused glass particles which produce varying pHs.. A similar project was previously conducted concerning the bacteria’s susceptibility to varying borate concentrations contained within glass particles. We were replicating the experiments with phosphate based glass with varying concentrations of phosphate which modified the pH of the environment. Through the compaction of silver infused glass particles of varying concentrations, compacted disks were applied to a field of bacteria of the aforementioned strains and allowed approximately a day of incubation before the area of affection was measured. Our results indicated that the silver embedded glass was most effective at killing the bacteria at a pH which is closest to neutral.
Further experimentation was to be performed with the three aforementioned bacterial strains using SC-ICP-MS and SP-ICP-MS to correlate the amount of silver required to inhibit a bacterial cell. This required precise quantification of the bacterial cell concentration for the application of SC-ICP-MS and SP-ICP-MS. Because staphylococcus bacteria grow in small clusters, we tested various methods to disrupt the clusters and to correlate turbidity with accurate cell concentration. Following treatment of the bacterial strains with silver nanoparticles, the specific amount of cells affected would be determined by measuring the living concentration of cells.