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The Antimicrobial Activity of Silicon Copper Nanoparticles

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Result of this Campus Sustainability Research study were used to support formal application by Dr. Kolasinski and Dr. Pisciotta to an external Charles E. Kaufman Foundation Integrated Research-Education Grant in 2021.

After graduating from WCU, Angela Mangano received a full academic scholarship to Rutgers University where she is working towards a Ph.D. degree in plant biology.

Abstract

Excessive overuse of antibiotics has led to the emergence of mutations within bacteria that result in drug resistance. In an effort to combat the expanding issue of drug resistance, nanoparticles' bactericidal capabilities are being researched as an alternative to antibiotics (Beyth, 2015). In this paper, research to explore a novel silicon copper nanoparticle's antimicrobial capabilities will be discussed. The novel nanoparticle was developed by West Chester University professor, Dr. Kolasinski. The specific research goals were to determine the time and dose dependency of the nanoparticles and investigate the antimicrobial spectrum of activity against Gram-positive bacteria, Gram-negative bacteria, and viruses. Gram-positive *Staphylococcus aureus* (*S. aureus*) and Gram-negative *Escherichia coli* (*E. coli*) were treated with 1 milligram and 10 milligrams of nanoparticle material for various time intervals to assess the amount of bacterial colony inhibition caused by each exposure time and dosage. It was observed that inhibition levels were highly time and dose-dependent. Further, *S. aureus* was found to be more susceptible to the nanoparticle treatment than *E. coli*. This research provides further supporting evidence of nanoparticle bactericidal efficacy and encourages the future utilization of nanoparticles as an antimicrobial substitution for antibiotic treatment.

Introduction

The antibacterial mechanisms employed by nanoparticles include the disruption of the bacterial cell membrane and interactions with proteins for intracellular antibacterial effects (Wang, 2017). In particular, copper nanoparticles can regulate metabolic processes of bacteria by acting on target proteins, resulting in the inhibition of nitrate and nitrite reductase (Wang, 2017). Nanoparticles' effectiveness at combatting drug resistance results from their application of more than one of these antibacterial mechanisms simultaneously (Sánchez-López, 2020). Since a pathogenic microbe is unlikely to have multiple mutations, it becomes more difficult to acquire resistance (Sánchez-López, 2020). Nanoparticles that interact with the bacterial cell wall continuously release ions, causing more toxicity to the cells. The large generated ion concentration further helps to penetrate the cells (Slavin, 2017). The research discussed in this paper investigated the bactericidal efficacy of copper nanoparticles coupled with silicon. It was hypothesized that there would be a positive correlation between the independent variables of nanoparticle dosage and time of exposure, and the inhibition of microbial colonies.

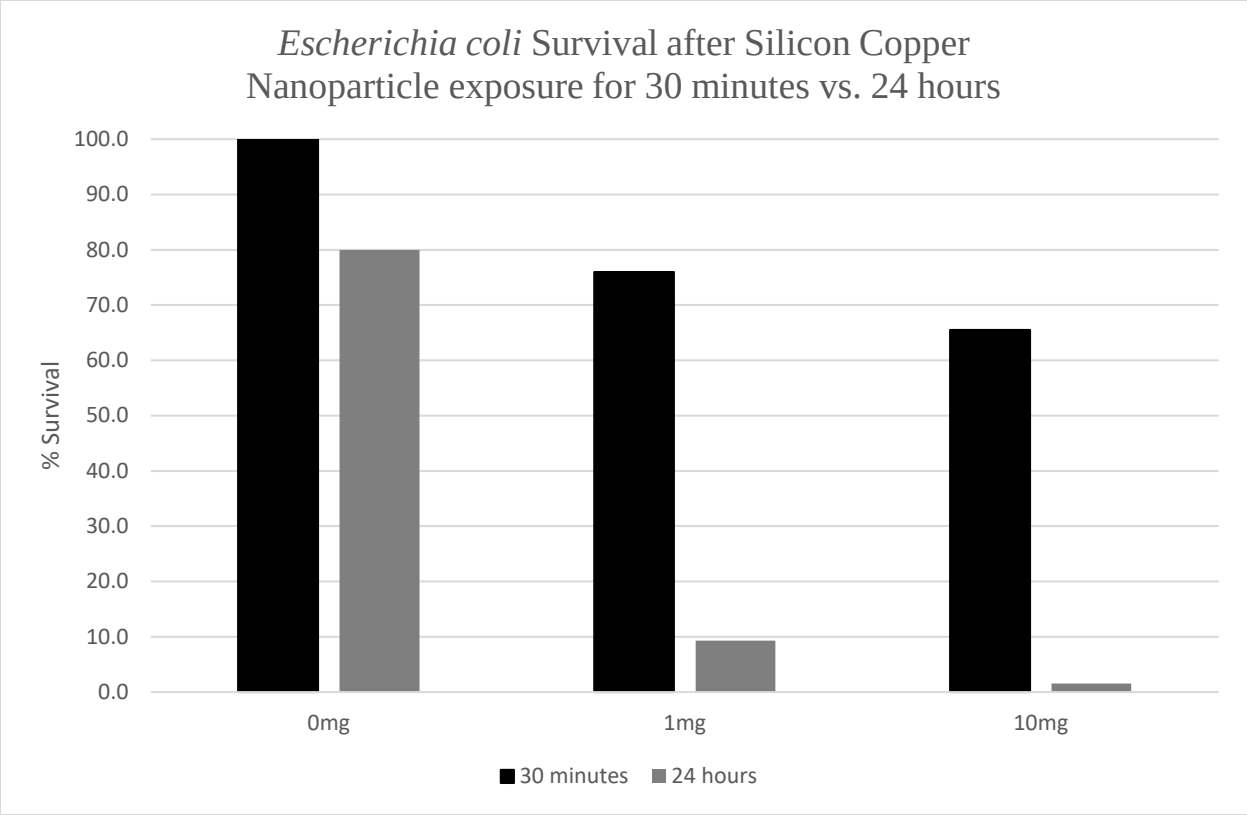
Methods and Materials

To confirm the bacterial culture's purity, a Gram stain was performed to ensure no cross-contamination had occurred between bacterial species. A buffer solution was then created by inoculating an isolated colony of bacteria into 4mL of deionized water. A spectrophotometer set to 600 nanometers was then used to obtain an optical density reading of 0.1. After this reading was obtained, 1mg and 10mg of nanoparticle material were measured and placed into two separate Eppendorf tubes. A third Eppendorf tube was prepared with 0 mg of nanoparticle material to serve as the control. 1 mL of the buffer solution was then added to all 3 Eppendorf tubes. The tubes were spun in a tube rotator to treat the bacteria with nanoparticle material for varying time intervals. *E. coli* was spun for 30 minutes and 24 hours, and *S. aureus* was spun for 30 minutes, 1 hour, 3 hours, and 24 hours. 100µL was taken from each Eppendorf tube to perform a dilution series to 10(-6) for each nanoparticle concentration. Each dilution was plated on tryptic soy agar (TSA) media and incubated for 48 hours.

In addition to the dilution series using bacteria, a T4 bacteriophage assay was performed using the overlay method. A viral dilution series was performed to reach the optimal number of colony-forming units (CFUs) to be infected by the T4 plaque-forming units (PFUs), which was calculated to be 1x10(8) CFUs/mL. Then, *E. coli* and 100µL of the virus were added to soft agar. After inverting the tubes, they were quickly poured over the bottom agar plate and incubated.

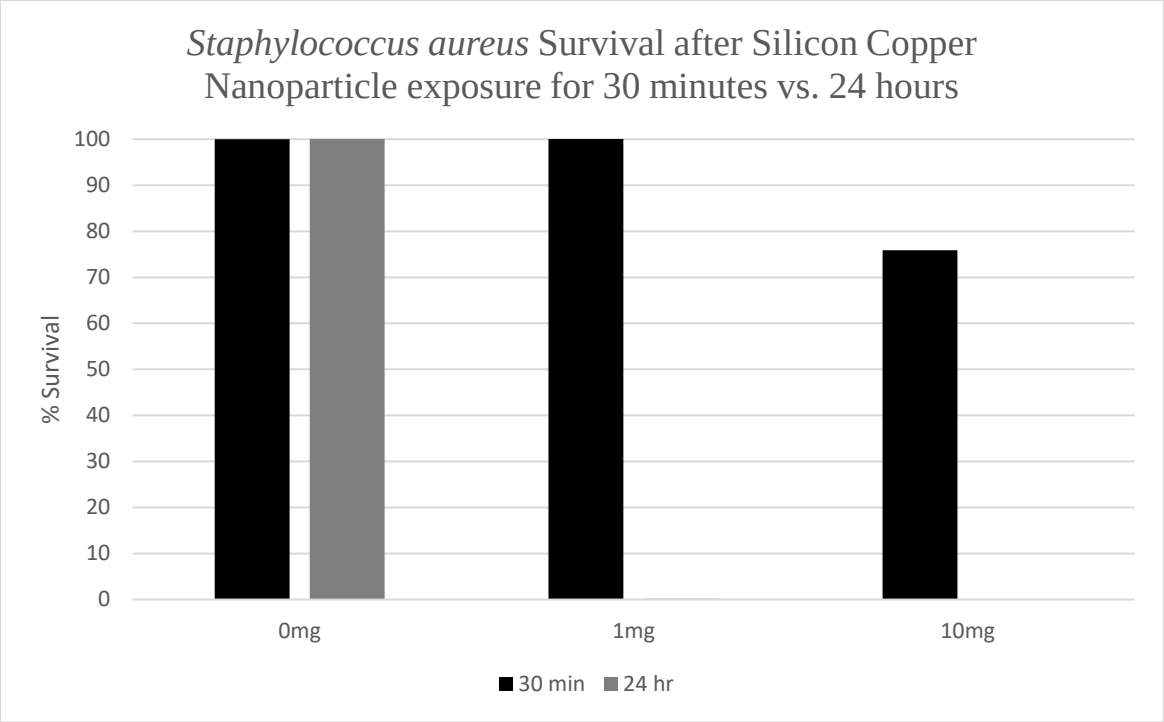
Results

The results of *E. coli* colony inhibition are shown below in Graph 1. What is observed is a 24% inhibition of colonies after 30 minutes of treatment with 1mg of silicon copper nanoparticle material, as compared to the control. After 24 hours of exposure, with the same nanoparticle dosage, 90.5% colony inhibition is observed. With a 10mg dosage, 35% colony inhibition was recorded after 30 minutes of exposure, and 98% of colonies were inhibited after 24 hours. This supports the hypothesis that time of exposure and dosage are heavily influential in observed inhibition levels.

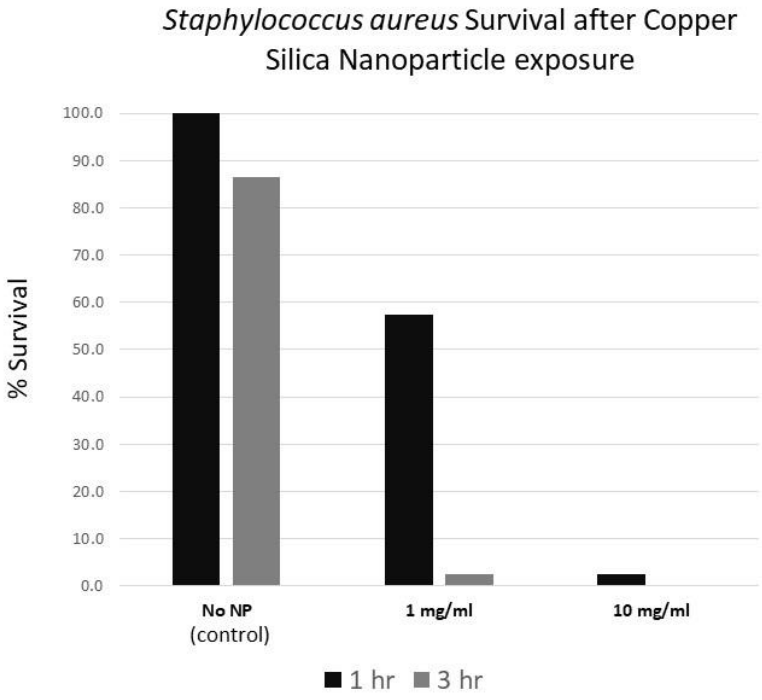


Graph 1: *E. coli* Survival after Silicon Copper Nanoparticle exposure for 30 minutes vs. 24 hours

The results of *S. aureus* colony inhibition after 30-minute treatment and 24-hour treatment, shown in Graph 2, exemplify a similar inhibition pattern noted in the *E. coli* trials. With a 1mg dosage, there was no inhibition after 30 minutes and 99% inhibition after 24-hour treatment. With the 10mg dosage, there was 24% colony inhibition after 30 minutes and complete inhibition after 24-hour treatment. For the 1-hour intermediate time interval, shown in Graph 3, 43% inhibition was recorded with 1mg of nanoparticle treatment, and 96% inhibition was noted with the 10mg nanoparticle treatment. For the 3-hour intermediate time interval, 97% of colonies were inhibited after treatment with 1mg, and complete inhibition was reached with 10mg nanoparticle treatment.



Graph 2: *S. aureus* Survival after Silicon Copper Nanoparticle exposure for 30 minutes vs. 24 hours



Graph 3: *S. aureus* Survival after Silicon Copper Nanoparticle exposure for 1 hour vs. 3 hours

The results of the T4 bacteriophage assay did not show virus inhibition after the 1-hour exposure to nanoparticles. This assay could be repeated using the 3-hour and 24-hour intervals, as successful bacterial inhibition was seen with those exposure times.

Discussion

The results of this experiment affirmed the aforementioned hypothesis that there is a positive correlation between nanoparticle dosage and time of exposure, and inhibition of microbial colonies. It was observed that inhibition levels were highly dependent on the exposure time, and the nanoparticles displayed high levels of dose-dependent efficacy. It was also observed that the Gram-positive *S. aureus* displayed higher susceptibility to nanoparticle treatment than the Gram-negative *E. coli* did. It would be worthwhile to investigate if all Gram-positive bacterial strains are more sensitive to nanoparticle treatment, or if variations in susceptibility are strain-specific.

Nanoparticles are being utilized in surface coatings, i.e., exercise equipment coatings. In one study by Dr. Pisciotta and Dr. Fan, ampicillin-resistant *Staphylococcus* was found on West Chester University's campus gym equipment. 43% of bacteria were resistant to ampicillin treatment, and 73% of those isolates were also resistant to multiple other drug treatments (ASM, 2020). This study reinforced the relevancy of finding solutions to combat the widespread issue of antibiotic resistance. Further, the research presented in this paper provides supporting evidence for the potential of nanoparticles to be used as one such solution in the future.

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