Letters in
General Microbiology

Effect of Copper Nanoparticles on Microbes

Rachel Rutecki

Department of Biology, Rutgers University, Camden NJ 08102

Abstract

Nanoparticles are particles with at least one dimension on the order of 100 nm or less; thus, they possess different properties than their bulk constituent, due to their high surface area to volume ratio. It has been suggested that the higher surface area of nanoparticles will further enhance the antimicrobial characteristics of certain bulk materials. This study seeks to determine possible antimicrobial effects of copper nanoparticles. We will observe what effects copper nanoparticles have on the growth and survival of bacteria, *Escherichia coli* and fungus, *Neurospora crassa*. The current study suggests that copper nanoparticles inhibit the germination of *N. crassa* and kill *E. coli*.

Introduction

Nanoparticles are particles with at least one dimension on the order of 100 nm or less; thus, they possess different properties than their bulk constituent, due to their high surface area to volume ratio. Several bulk metals are known as antimicrobial materials; for example, silver and copper are thought to react with proteins, combining the disulfide bonds of enzymes, thus inactivating the proteins of microbes. It is suggested that the higher surface area of nanoparticles will further enhance the antimicrobial characteristics of certain materials.

Previous studies have looked into nanoparticles of several compositions to gauge their antimicrobial properties. One study used engineered PEGylated micellar nanoparticles to inhibit the growth and toxicity of multiple myeloma cells. This study suggested that the internalization of the nanoparticles by MM cells induced DNA double-strand breaks and apoptosis, while also overcoming cell adhesion mediated drug resistance, with increased efficacy (Kiziltepe 2012). Another study developed a mullite-based antimicrobial ceramic composite with the adsorption of a copper nanoparticle suspension, which demonstrated over 99% mortality for several bacterial species after 24 hours of incubation (Bagchi 2012). Further studies have suggested that copper nanoparticles inhibit the activity of bacteria more than that of fungus (Ramyadevi 2012).

Studies have also questioned what factors influence the success of the antimicrobial agents. It has been stated that the toxic effect of copper nanoparticles on microbes depends on several factors: pH, temperature, aeration, concentration of the microbe, concentration of nanoparticles, and the interactions among these parameters (Rispoli 2010). Bacteria were more sensitive to copper nanoparticles than silver nanoparticles, suggesting that a more effective antimicrobial approach would prefer copper nanoparticles over silver (Yoon 2007). Copper has also been preferred over silver because of its lower cost, and possibly less toxic effects on the host than silver. Different nanocomposites studied have exhibited varied antimicrobial behavior. One study showed copper and copper oxide nanoparticle composites that, after four hours of contact, killed over 95% of the bacteria; this study attributes the antimicrobial behavior to Cu2+ released from the bulk of the composite (Delgado 2011). Copper oxide nanoparticles showed antimicrobial activity against several pathogens in another study, enhanced with the presence of sub-minimum bactericidal concentrations of silver nanoparticles. This study further suggests that the release of ions may be necessary to microbicidal functions (Ren 2009).

This study seeks to determine possible antimicrobial effects of copper nanoparticles. Here, we will observe what effects copper nanoparticles will have on the growth and survival of *E. coli* and *N. crassa*.

Materials & Methods

Fungal Study:

The fungus used in this study was *N. crassa* strain L1 (Lab strain, KL). Nanoparticles used in this study were purchased from SkySpring Nanomaterials, Inc. Copper nanoparticles of size=500 nm and size=40-60 nm were used; 500 nm copper nanoparticles were used in trials expressed in the Results section, whereas 40-60 nm copper nanoparticles were used in previous, inconclusive trials used to optimize research techniques. The process described below explains how we obtained the data represented in the Results section.

N. crassa was grown for one week prior to its use. Glass microscope slides were cleaned with 70% Ethanol before

melted LB media was poured onto the slides in a thin layer. A conidial suspension was made of the one week old *N. crassa*; from this, four sample types were made. (1) Control sample; conidial suspension of 1x concentration in sterile water. (2) Control sample, conidial suspension of 5x concentration in sterile water. (3) NP 1x sample, conidial suspension in sterile water with 1x concentration of copper nanoparticles from stock solution. (4) NP 5x sample, conidial suspension in sterile water with 5x concentration of copper nanoparticles from stock solution. Each sample type was pipetted onto slides with melted media, at opposing ends of two prepared slides. Each of the four sample types were observed when prepared, approximately 0.5 hours after the conidial suspension was prepared and added to the media. Each sample was observed again 3 hours after being prepared. Between 0.5 and 3 hours, samples were stored at 30 Celsius, suspended in sterile water within a petri dish, to avoid drying out.

Samples of *N.* crassa, with and without copper nanoparticles, were observed at 0.5 and 3 hours after being prepared. The number of spores and germinated spores were visually counted. The images of germinating conidia were recorded using Canon Rebel XT with DSLR Remote Pro and Zeiss Universal Microscope with P1 232nm 28 adapter. The photographs were analyzed using ImageJ. The percent germinated was determined by:

$$\% \text{ Germinated} = Ng/(Ng+Nc)$$

where Nc is the number of conidia that had not germinated, and Ng is the number that had germinated.

Bacterial Study:

The bacteria used in this study was *E. coli* pUC19. Nanoparticles used in this study were purchased from SkySpring Nanomaterials, Inc. Copper nanoparticles of size=500 nm and size=40-60 nm were used; 500 nm copper nanoparticles were used in trials expressed in the Results section, whereas 40-60 nm copper nanoparticles were used in previous, inconclusive trials used to optimize research techniques. *Pseudomonas syringae* (DC3000) was also used towards improving research techniques, but yielded inconclusive data. The process described below explains how we obtained the data represented in the Results section.

E. coli was streaked onto LB media plates. Three new LB plates were later inoculated from the streak plate, with three inoculation sites on each plate. The three inoculation sites on each plate were of the following sample type: (1) Control sample, 10 µl sterile water pipetted onto the inoculation site; (2) NP 1x sample, 10 µl of 1x concentration of copper nanoparticle solution pipetted onto the inoculation site; (3) NP 5x sample, 10 µl of 5x concentration of copper nanoparticle solution pipetted onto the inoculation site. Plates were observed for six days, and the diameter of each site was measured (diameter, in mm). Photographs were taken of each plate over the course of six days for visual analysis of each sample type.

Results

Samples prepared with conidial suspensions of *N. crassa*, with and without nanoparticles, were observed at 0.5 hr and 3 hr after preparation. At t=0.5 hr, the three sample types (control conidial suspension without nanoparticles; 1x concentration of copper nanoparticles in conidial suspension; 5x concentration of copper nanoparticles in conidial suspension) had the same average percentage of germination (P values as follows: Control vs. 1x: 0.79; Control vs. 5x: 0.32; 1x vs. 5x: 0.32). At t=3 hr, the percentage of conidia that had germinated was highest in the control sample, followed by the 1x concentration of copper nanoparticles, and lastly, the 5x concentration of copper nanoparticles (see Fig. 1). This suggests that the higher concentration of copper nanoparticles most effectively inhibits the germination of *N. crassa*. However, the results did not show a statistical difference (P values were above 0.05); thus we are unable to reject the null hypothesis with confidence.

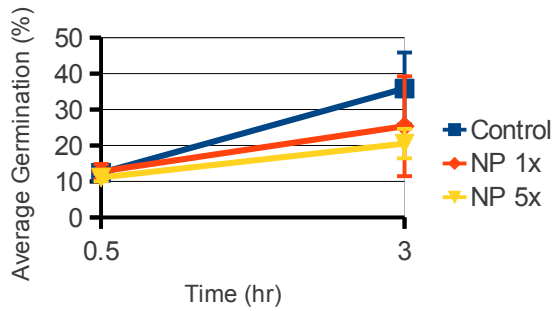

Figure 1: Effects of Copper Nanoparticles on *N. crassa.* Average percent germination of L1 after inoculation. Sample types: control, 1x concentration of copper nanoparticles, 5x concentration of copper nanoparticles. Error bars represent standard deviation.

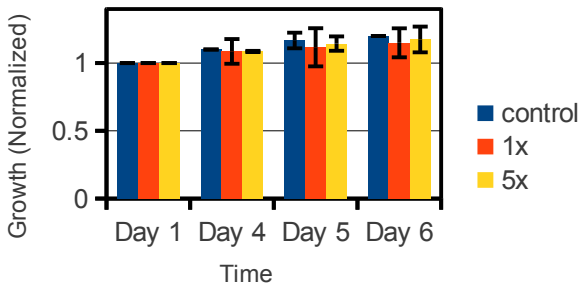

Figure 2. Effects of Copper Nanoparticles on *E.* coli. Normalized growth rate of E. coli over six days. Sample types: control, 1x concentration of copper nanoparticles, 5x concentration of copper nanoparticles. Error bars represent standard deviation.

Plates inoculated with *E. coli*, with and without nanoparticles, were observed over the course of six days. According to Fig. 2, the diameter of growth was the same among the sample types (control inoculation without copper nanoparticles; inoculation with 1x concentration of copper nanoparticles; inoculation with 5x concentration of copper nanoparticles). However, by visual analysis of Fig. 3, we see a difference in the quality of the three conditions at day 5 (Fig. 3b). The control condition at day 5 appears to be healthy, whereas the inoculations with copper nanoparticles (1x and 5x) do not appear viable. Similar results to those shown in Fig. 3 were expressed in all three plates observed.

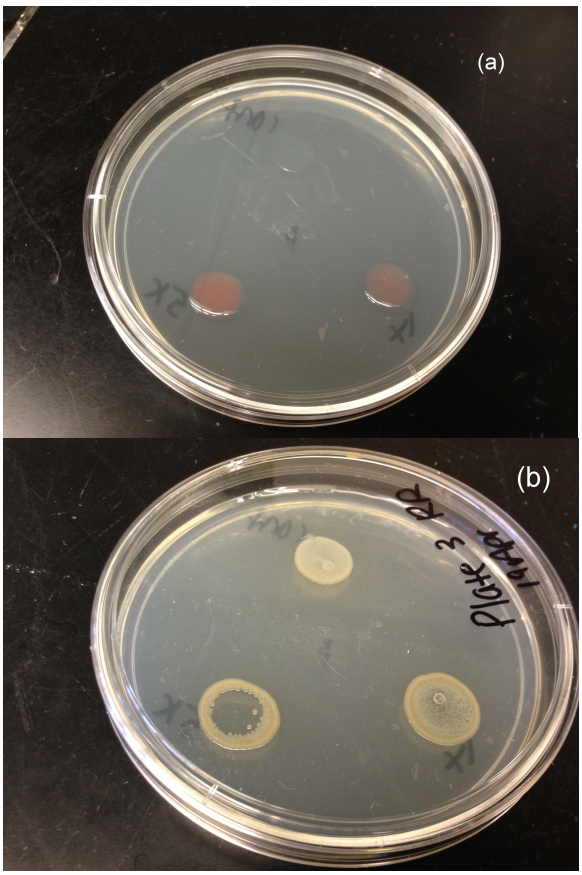

Figure 3. (a) *E. coli* with control, 1x concentration of nanoparticles, and 5x concentration of nanoparticles (Day 1). (b) *E. coli* with control, 1x concentration of nanoparticles, and 5x concentration of nanoparticles (Day 5).

Discussion

Fungal Study:
This study was designed to test the effect of copper nanoparticles on *N. crassa*. The results suggest that copper nanoparticles negatively affect *N. crassa*, inhibiting its germination. However, statistical analysis of the results of this study cannot reject the null hypothesis with confidence. Similar studies could be conducted in the future using more samples, examined at more time intervals, to express more conclusive results.

Bacterial Study:
This study was designed to test the effect of copper nanoparticles on *E. coli*. The results show that the viability of *E. coli* is impaired by the presence of copper nanoparticles, while the diameter of growth of *E. coli* is unaffected. Further studies could be done with similar samples, inoculating new plates from each of the three conditions after several days. This would determine if the samples of *E. coli* with copper nanoparticles, which appear dead, are able to stimulate new growth. A study could also be done to see if the *E. coli* cells on the outermost section of the sample have developed a resistance to certain concentrations of copper nanoparticles.

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