

Antimicrobial Effects of Metal Oxide Nanoparticles

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Abstract and Introduction:

In a world of emerging nanotechnology, one of the primary concerns is the potential environmental impact of nanoparticles (NPs). An efficient way to estimate nanotoxicity is to monitor the response of bacteria exposed to these particles [1]. This experiment explored the antimicrobial properties of nickel oxide, cobalt (II,III) oxide, zinc oxide, copper (II) oxide, iron (III) oxide, titanium dioxide, and iron (II,III) oxide against a model microorganism, *Escherichia coli*. The toxicity of these metal oxide NPs was tested using two methods: culturing in liquid media containing NPs, and electrospraying the NPs directly onto bacterial surface.

Aqueous exposure mimics the natural interaction between microbial species as NPs diffuse in the environment [2]. During these tests, there was noticeable aggregation, preventing effective interaction between the particles and the bacteria. The limited growth inhibition observed from this form of exposure to metal oxide NPs was therefore attributed to their ionic species.

On the other hand, the electrospray technique allows direct interaction between the NPs and cells. This exposure method grants insight into how “nano” associated properties from metal NPs affect the environment [2]. This method observed a higher death rate when the bacteria were exposed to oxidized nickel, zinc, and cobalt species; but no antimicrobial properties from titanium or iron. The disparity in the results of the two exposure techniques indicates that toxicity is dependant both upon the exposure method and the size of the particle.

Experimental Procedure:

Escherichia coli (*E. coli*) were cultivated in M9 minimal media at 37°C. Optical density was measured at 600 nm (OD₆₀₀) using a UV spectrometer (Genesys, Thermo-Scientific, USA). Experiments began with a 5 mL *E. coli* culture with OD₆₀₀ = 0.05 in M9 minimal media. The aqueous exposure method tracked the growth rate of *E. coli* with 2,

NP	Aqueous	Electrospray	Ionic
CuO	NT	N/A	T>2µg
NiO	NT	T	T>2µg
Co3O4	NT	T	T>2µg
ZnO	NT	T	NT
Fe2O3	NT	NT	NT
Fe3O4	NT	NT	NT
TiO2	NT	NT	NT

Table 1: Summary of results. T= toxic, NT = non-toxic.

20, and 100 mg/L NPs. OD₆₀₀ was recorded at 3, 6, 9, 21, 24 hours. The experiment was also performed using equivalent amounts of soluble chloride salt of the metals to test ionic toxicity.

For electrospray exposure experiments, the aliquot of *E. coli* was first filtered onto a polyvinylidene fluoride (PVDF) membrane (0.22 µm pore size, 1.25 cm × 1.25 cm, Millipore, US) to form a biofilm, which was then electrosprayed with NPs. The electrospray system was kept at a flow rate of 5 µL/min and a current of ~ 7 kV to maintain a cone shaped spray; the particles were suspended in 1.0 M sodium phosphate (Na₂HPO₄, pH 7) buffer. Then the biofilm was washed from membrane using minimal medium and the total living cells after electrospray exposure was measured based on the colony forming unit (CFU) using LB agar plates. Colonies were counted after the plates were incubated in 37°C for ~ 24 hours. Meanwhile, the cells from biofilm will be resuspended in the M9 minimal media and the recovery of growth was monitored by OD₆₀₀. Scanning electron microscopy (SEM) was used to observe changes in cell morphology after exposure to NPs.

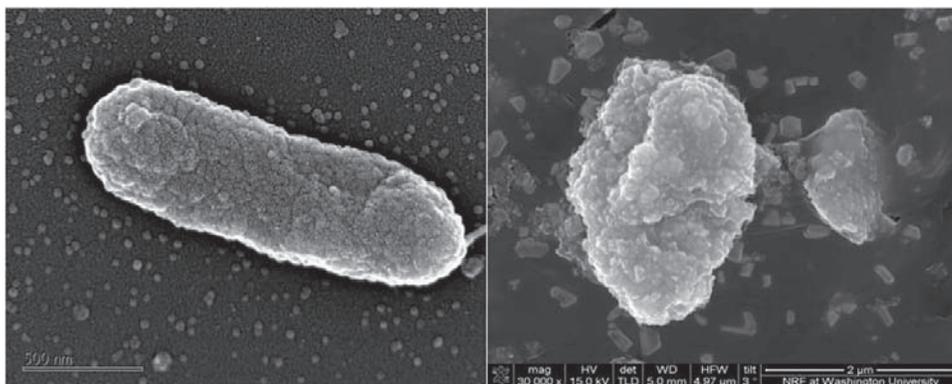


Figure 1: Comparison of an undamaged *E. coli* cell with one that has been electrospayed with nickel oxide.

electrical field, ionic zinc, and buffer were nontoxic. The next two bars show the inhibition when electrospayed with 4 $\mu\text{g/L}$ and 20 $\mu\text{g/L}$ zinc oxide NPs. This confirmed concentration has direct effect on toxicity. Next, the CFU after electrospaying with 4 $\mu\text{g/L}$ zinc oxide microparticles (480 nm diameter) confirms toxicity increases as particle size decreases. Finally, the CFU of *E. coli* exposed to titanium dioxide was used as a reference between zinc oxide and a nontoxic metal oxide.

Results and Conclusions:

The growth curves from the aqueous exposure method displayed no growth inhibition from NPs, because all NPs aggregated. All ionic species, excluding iron and titanium, were inhibitory above 2 $\mu\text{g/L}$. Growth curves show that the electrospay exposure method was able to cause significant cell death when *E. coli* was exposed directly to nickel oxide, cobalt (II, III) oxide, and zinc oxide (nickel oxide shows highest toxicity). The *E. coli* grew more efficiently and consistently when electrospayed with iron oxide NPs, were unaffected by titanium dioxide NPs, and copper (II) oxide was unclear. These results are summarized in Table 1. Figure 1 compares an undamaged *E. coli* cell with one that has been electrospayed with nickel oxide.

Zinc oxide exposure was tested more thoroughly than the other metal oxides and the inhibition from electrospay exposure was clearly observed as function of doses and sizes. Figure 2 shows the increase in recovery time after electrospaying (solid square), as opposed to recovery from aqueous exposure (diamond), and uninhibited growth (open square). The complete results from zinc oxide testing can be seen in Figure 3. From left to right, the non-sprayed bacteria show a similar CFU to those electrospayed with water, zinc chloride, and sodium phosphate buffer. This confirmed the

Future Work:

The CFU data collected from some metal oxide NPs was too inconsistent for conclusions. We will repeat these experiments and collect CFU data after electrospaying. Tunneling electron microscopy (TEM) images have been suggested to identify whether NPs entered into cells after electrospaying.

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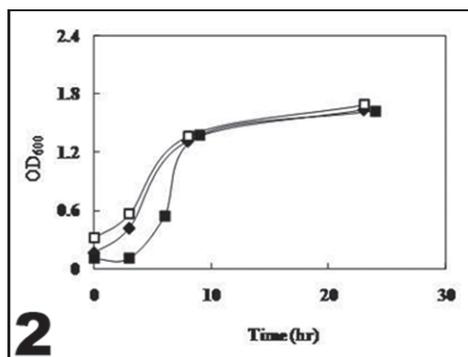


Figure 2: Increase in recovery time after electrospaying (solid square), as opposed to recovery from aqueous exposure (diamond), and uninhibited growth (open square).

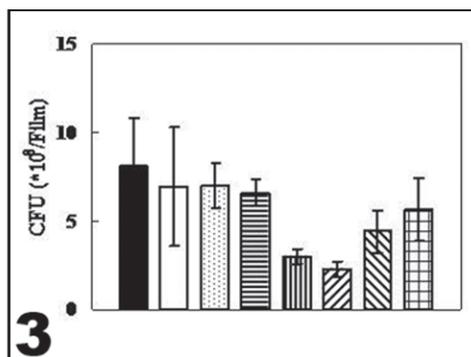


Figure 3: Complete results from zinc oxide testing.