Co-Selection of Metal- and Antibiotic-Resistance Following Nanoparticle Exposure

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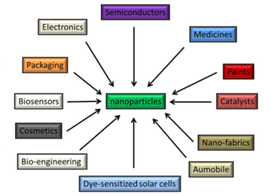
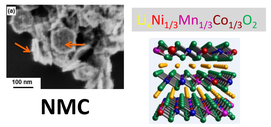
Introduction

Nanoparticles- Structure and Applications

- Nanoparticles are materials between 1 and 100 nanometers, which promote a very large surface-area-to-volume ratio[1]
- Nanoparticles also boast a variety of size-dependent, tunable properties such as color, supermagnetism, and quantum confinement in semiconductor particles[1]
- Produces unique chemical properties not afforded by the same material in bulk, such as increasing the rates of chemical reactions[1]
- The lithiated nickel manganese cobalt (NMC) nanoparticle (pictured right) is used in lithium-ion hybrid car batteries, little is currently known about environmental impacts[2]
- Nanoparticles composed of heavy metals (lead, tin) are highly stable and do not break down easily, and thus can cause toxic effects in the environment[1]



Mbonyiryivuze, Agnes, et al. "Titanium Dioxide Nanoparticles Biosynthesis for Dye Sensitized Solar Cells application: Review." *Physics and Materials Chemistry* 3.1 (**2015**): 12-17.

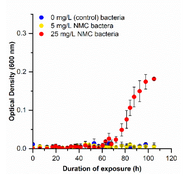


NMC

Voeikova, Tatiyana et al. *Applied Biochemistry and Microbiology* 52 (**2015**): 769-775.

The Problem

- Recently, it has been found that nanoparticles can cause resistance in bacteria
  - Resistance occurs when bacteria adapt or change (often through mutations in DNA) to withstand a substance that is normally harmful to the organism[3]
- According to a study using the bacteria *Shewanella oneidensis* and a lithiated nickel manganese cobalt oxide (NMC) nanoparticle, bacterial exposure to NMC at a concentration of 5 mg/L substantially hindered bacterial population growth and respiration[1]
- An indicator of bacterial resistance is an increased minimum inhibitory concentration (MIC, the lowest concentration of a chemical which hinders visible growth of bacteria), meaning that the bacteria can survive given higher concentrations of the toxic nanoparticle[3]



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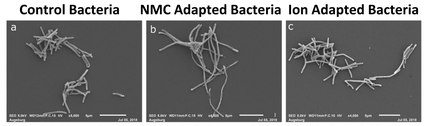
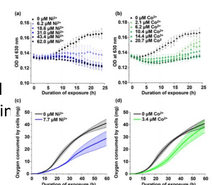
Control Bacteria **NMC Adapted Bacteria** **Ion Adapted Bacteria**



Mitchell, S. L.; Hudson-Smith, N. V.; Cahill, M. S.; Reynolds, B. N.; Frand, S. D.; Green, C. M.; Wang, C.; Hang, M. N.; Hernandez, R. T.; Hamers, R. J.; Feng, Z. V.; Haynes, C. L.; Carlson, E. E. Chronic Exposure to Complex Metal Oxide Nanoparticles Elicits Rapid Resistance in Shewanella Oneidensis MR-1. *Chemical Science* **2019**, 10 (42), 9768–9781.

Background and Goals

- A good method for modeling the effect of nanoparticles on the environment is through bacterial exposure to nanoparticles
- Bacteria are members of the lowest trophic level, control nutrient cycling in the environment, respond to changes in their environment, and replicate rapidly[3]
- *Shewanella oneidensis* is the ideal bacterial candidate because it can survive in both anaerobic and aerobic conditions, recycles minerals in soil, and is widely distributed around the globe[2].
- The goal is to find the MIC of a panel of antibiotics with different mechanisms of action, or specific biochemical interactions that dictate how a drug produces its effect[4]
- The mechanisms of action of the antibiotics will suggest which metabolic/developmental processes of the bacteria are affected by the nanoparticles, inducing resistance[5].



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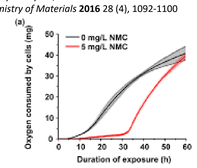

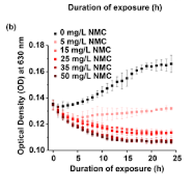

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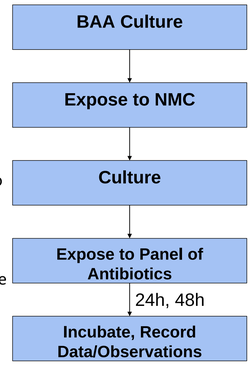
Goals of the Project

- Seeking to understand bacterial resistance to nanoparticles in a variety of ways, specifically discovering if there is a correlation between bacterial resistance to the NMC nanoparticle and antibiotics.
- Find the MIC of a panel of antibiotics with different mechanisms of action, or specific biochemical interactions that dictate how a drug produces its effect
- Examine the potential effects of NMC nanoparticles on living organisms within the natural environment in years to come

Process

- Preliminary colony counting experiments were conducted to estimate the average amount of *Shewanella oneidensis* colonies that would grow from 2 µL and 5 µL spots of bacteria culture, data was recorded
- A group of the wild type *Shewanella oneidensis* (BAA) was exposed to NMC nanoparticles, cultured, and the process was repeated several times to create a resistant strain of the bacteria
- Both the control (BAA) bacteria and the NMC resistant bacteria were streaked onto agar plates, incubated, and cultured in preparation for the following antibiotic assays
- A range of concentrations of each chosen antibiotic were prepared from a stock concentration and pipetted into 25 mL molten LB-agar plates divided in half- for the control and NMC resistant strains respectively- and left to cool overnight
- The NMC and BAA bacteria were cultured and pippeted, using 6 5µL drops, onto the agar plates under a fume hood
- Results were recorded at 24 and 48 hour checkpoints



Results

Erythromycin

Control	
Concentration (mg/L)	Growth Level
1	MG
2	SG
3	SG
4	NG
5	NG
6	NG

NMC		
Concentration (mg/L)	Dilution	
	1:100	1:1000
6	HG	SG
12	HG	SG
18	SG	SG
24	SG	SG
30	SG	SG
36	NG	NG
48	NG	NG
64	NG	NG
80	NG	NG
90	NG	NG
100	NG	NG

Key	
NG	No growth
SG	Slight growth
MG	Medium growth
HG	Heavy growth

Kanamycin

Control	
Concentration (mg/L)	Growth Level
4	MG
8	SG
10	NG
12	NG
14	NG
16	NG
18	NG
22	NG
26	NG
28	NG

NMC		
Concentration (mg/L)	Growth Level	
100	HG	MG
120	MG	MG
140	MG	MG
160	MG	MG
180	SG	SG
200	SG	SG

Metronidazole

Concentration (mg/L)	Control	NMC
5	MG	HG
10	SG	HG
15	SG	HG
20	SG	HG
30	SG	HG
40	SG	HG
50	SG	HG
60	SG	HG
80	NG	HG
100	NG	HG
120	NG	HG
140	NG	HG

Novobiocin

Concentration (mg/L)	Control	NMC
5	HG	MG
10	MG	SG
15	SG	SG
20	SG	SG
30	SG	SG
40	NG	NG
50	NG	NG
60	NG	NG
80	NG	NG
100	NG	NG
120	NG	NG
140	NG	NG

Puromycin

Concentration (mg/L)	Control	NMC
0.5	HG	HG
1	HG	HG
2	MG	HG
4	MG	HG
8	MG	HG
16	MG	MG
32	MG	MG
64	SG	SG
128	SG	SG

Analysis

- The results of the assay experiments on the 5 different antibiotics show that generally, the NMC resistant *S. oneidensis* increased resistance to the antibiotics
- The largest and most consistent increases in resistance for the NMC strain of bacteria was seen in the antibiotics erythromycin and kanamycin, which indicates that the mode of bacterial resistance could involve protein synthesis or DNA synthesis.
- Several trials were inconclusive, suggesting that either the antibiotics were inactive, contamination occurred, or that they are not compatible with *S. oneidensis.*

Antibiotic	Mechanism of Action
Erythromycin	Inhibits protein synthesis
Kanamycin	Inhibits DNA synthesis
Novobiocin	Inhibits rRNA synthesis
Metronidazole	Inhibits DNA synthesis
Puromycin	Inhibits protein synthesis

Conclusion

- The most effective antibiotics at inducing NMC resistance in *Shewanella oneidensis* were shown to be kanamycin (MICs 8-10 mg/L for control and 160-180 mg/L for NMC) and erythromycin (MICs 3-4 mg/L for control and 30-36 mg/L for NMC)
- Due to the protein filamentation, or elongation, of the NMC-resistant strain of *S. oneidensis* after exposure to NMC, it can be concluded that the process of nanoparticle-resistance induces some type of metabolic stress within living organisms.
- An interesting extension would be to conduct MR spectroscopy on the S. oneidensis to learn more about its structure and processes.
- Beneficial next steps would be to test a wider array of antibiotics with more diverse mechanisms of action (MOA), as well as repeat trials with antibiotics of similar MOAs- namely protein synthesis and DNA synthesis.
- NMC nanoparticles are noted to induce resistance in gram-negative bacteria, and therefore have the potential to disrupt ecosystems and other environmental aspects.

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