Antimicrobial Chiral Cerium Oxide Nanoparticles

ENGINEERING HONORS PROGRAM
UNIVERSITY OF MICHIGAN

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Antibiotic Resistant Microbes

Every year in the United States, more than 35,000 people die because of infections caused by antibiotic-resistant bacteria, with more than 2.8 million infections occurring annually[1]. Globally, more than 700,000 deaths are attributed to antimicrobial resistant infections each year (likely undercounted), and it is projected that by 2050 that number will rise to **10 million deaths per year**, more than currently die from cancer[2].

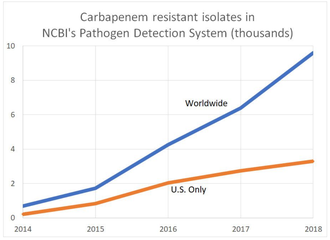

Figure 1. Increasing resistance to carbapenems observed across numerous pathogens in both the U.S. and worldwide[3].

Concerningly, the emergence of newly resistant pathogens is outpacing the development and implementation of new antibiotic treatments. Due to slow development of antibiotics, some countries are using last-line antibiotics at high rates, limiting their effectiveness for the future. **This indicates the desperate need for research and development of a new class of antibiotics.**

Antimicrobial Nanomaterials

To combat the looming antimicrobial resistance crisis, much work has been dedicated to research discovering and developing **nanomaterials as next generation antimicrobials**, defined as materials having at least one dimension measuring 1–100 nm. Nanomaterials have the potential to revolutionize the medical field, and many applications of nanomedicine have been proven.

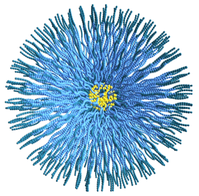

Figure 2. Nanoparticle with surface coated with long-chain ligands[4].

For example, lipid nanoparticles are used to encapsulate the mRNA used in the COVID-19 vaccine. Other applications of nanoparticles (NPs) include targeted drug and gene delivery, antimicrobial coatings, bacterial detection systems, and bactericidal vaccines. The exact antibacterial mechanisms of NPs are poorly understood, however it is common for NPs to exhibit multiple mechanisms simultaneously. Since resistance to these NPs would require multiple mutations, it is very difficult for bacteria to develop resistance to these materials.

Since the properties of NPs can vary greatly depending upon the size, shape, charge, and chemical composition, research into these materials has the potential to **dramatically expand the number of available antibiotics.**

Inorganic Nanoparticle Biomimicry

Biomimicry is the emulation of natural elements in the development of artificial systems and processes. This project focuses on biomimicry as a potential way to increase the interaction between inorganic NPs and biological systems such bacterial cells and biofilms.

One of the rapidly expanding fields of nanoscience and technology is chiral inorganic nanostructures. A molecule is chiral if it cannot be superimposed on its mirror image. Chiral NPs gained interest due to the the unusually strong circular dichroism (CD) observed for both individual NPs and their assemblies. NPs can be made chiral by either exposing them to circularly polarized light or by attaching chiral molecules to the surface of the NP core.

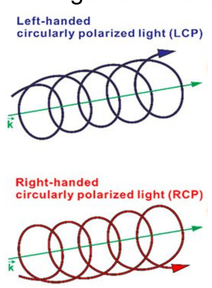

Figure 3. Chiral molecules differentially absorb left- and right-handed circularly polarized light[5]

Nanoparticle Synthesis Method

Cerium oxide nanoparticles (CeONPs) were selected as the inorganic nanoparticle to be investigated for this project.

- CeONPs were synthesized via the wet precipitation method
- Performed at room temperature conditions under moderate agitation
- Sodium citrate used as a chelating agent and sodium borohydride used as a reducing agent
- Four amino acid ligands examined (so far), cysteine, alanine, glutamic acid, and aspartic acid
- Reactions progressed for 2 hours before being stopped and “washed” using isopropanol. Centrifuged to obtain gel-like NP pellet that was re-dispersed in water and freeze-dried to obtain NP powders

Nanoparticle Analysis Methods

- Chiroptical activity of CeONPs was examined using circular dichroism
- Surface charge and dynamic light scattering size distribution profile determined using Zetasizer
- Exact size and morphology of CeONPs determined using transmission and scanning electron microscopy (TEM & SEM)

Experiments examining antimicrobial activity in planktonic bacteria cultures were conducted in 96 well plates filled with OD 0.01 bacteria and varying concentrations of CeONPs that were allowed to grow for 18 hours. Growth of bacteria was observed using plate readers. The bacteria we have examined (so far) are *E. coli* (UTI89) and *S. aureus* (USA300). USA300 is a strain of MRSA, which is antibiotic resistant.

Antimicrobial Potential

The results of a typical growth curve experiment for both *S. aureus* and *E. coli* are shown below. CeONPs are ineffective against *E. coli* but do show significant killing activity against *S. aureus*.

Another interesting observation from these experiments is that CeONPs exhibit varying levels of antibacterial activity depending upon the amino acid ligand used. We observe that the order of killing activity follows: Glutamate > Alanine > Cysteine > Aspartate. We also observed that L amino acids exhibited greater killing activity than D amino acids.

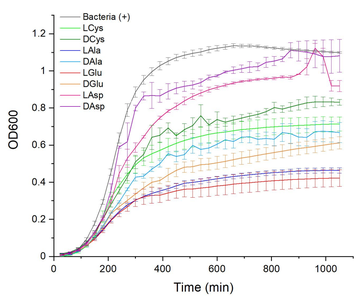

Figure 4. Results of a single growth curve experiment using *S. aureus*. Error bars represent standard error (n = 3).

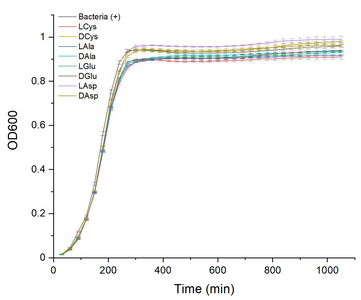

Figure 5. Results of a single growth curve experiment using *E. coli*. Error bars represent standard error (n = 3).

Other Nanoparticle Characteristics

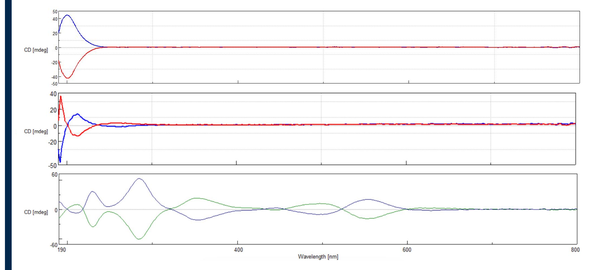

Figure 6. CD spectra of L/D cysteine (top), CeONPs with L/D cysteine (middle), and CeONPs doped with cobalt with L/D cysteine (bottom).

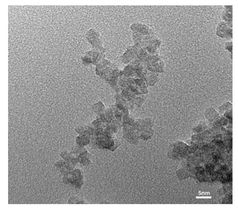

Figure 7. TEM image of CeONPs. Diameter of NPs around 5nm. *Credit: Dr. Sumeyra Turali-Emre.*

- Sintering at room temperature and pressure
- **Doping of cobalt into CeONP crystal structure**
- Attachment to aramid nanofibers to enhance strength (> 500%)

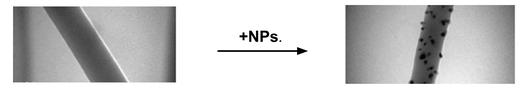

Figure 8. Addition of NPs to the surface of nanofibers can enhance their properties[6].

- Possible chiro-magnetism to induce and enhance chiroptical activity
- Sensitive to reaction pH

Figure 9. Demonstration of reaction sensitivity to pH indicated by color change.. From left to right: 0.5, 2, 5, 7, 9, 12, 14.

Conclusions and Future Work

Successfully developed facile synthesis method of bactericidal CeONPs. These NPs also exhibit a range of unique physical properties, making them an intriguing NP for further research.

- Investigate effect of pH, temperature, and removing citrate
- Further investigate the effect of doping structure with cobalt
- Investigate and quantify ability to prevent and disrupt biofilms
- Investigate and quantify cytotoxicity to mammalian cells
- Investigate more classes of amino acids
- Explore alternative quantifications of bactericidicity (e.g., ZOI)

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