Zirconium Oxide: A Nanoparticle for Antimicrobial Agents?

By Robert Lynn
Introduction/Background

• The antibiotic resistance crisis
  • Replication
  • Transcription
  • Translation

• Why? and how?
  • Fast reproduction times
  • Large population sizes
  • Transformation and conjugation
  • Inactivation and alteration

The medical concern

- Study by Huerta et al quantified the annual multidrug-resistant *Staphylococcus aureus* infections.
  - Cases increased from 127,000 to 278,000 over a span of six years from 1999 to 2005$^1$.
  - Annual MRSA related deaths increased from 11,000 to 17,000$^1$.
  - Although cases have decreased, antibiotic resistance is a serious concern.
A Possible Solution

- Metal-based antimicrobials (MBAs)
  - Target multiple cellular processes simultaneously
  - Metal oxide nanoparticles are effective antimicrobial agents
- Zinc oxide nanoparticles generate reactive oxygen species (ROS)
  - Damage cellular proteins, lipids, and DNA

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0209020
Experiment and Goals

• The study investigates zirconium oxide (ZrO$_2$) for possible antimicrobial activity

• Justification
  • Like Zinc, zirconium is a heavy metal
  • It can slowly release metal ions capable of crossing membranes and disrupting cellular processes from within the cell$^3$.
  • A study by Zahra Arefian et al verified ZrO$_2$ decreases activity of antioxidant enzymes such as Catalase, Glutathione Peroxidase and Superoxide Dismutase$^4$.
  • Synthesized as very small nanoparticles (4-10nm range)
Methods

- Zinc Oxide Synthesis
  - Solvothermal method from Zak Khorsand et al
  - Zinc acetate solution was prepared
  - Triethanolamine (TEA) was used as a polymerization agent
  - Solution stirred at 60°C for 1 hour
  - Cool to Room Temperature and placed in oven at 150°C for 18 hours
  - Ethanol washes and dried in the oven overnight at 70°C
Methods

• Zirconium Oxide Synthesis
  • Following Andrea Pucci et al, synthesis occurred under an inert argon atmosphere in the glovebox
  • Benzyl alcohol was purged of oxygen
  • In the glovebox, Zirconium (IV) isopropoxide isopropyl alcohol complex was added with the benzyl alcohol into a Teflon vessel
  • Vessel was placed in oven at 230°C for 48 hours
  • Precipitate washed with ethanol and acetone
  • A pellet centrifuged at 42,000 x g for 30 mins
  • Solid dried at 60°C overnight
Methods

- Characterization of nanoparticles
  - ZnO and ZrO$_2$ products verified with UV-Vis spectroscopy
  - Powder nanoparticles suspended in MilliQ water
  - An Abs vs. Wavelength spectra with 20 average scans
  - First derivative of absorbance with respect to energy used to find band-gap energy
Methods

• Antimicrobial Tests
  • Kirby Bauer performed using agar disc diffusion
  • Bacterial cultures inoculated in TSB using a sterile inoculation loop
    • *Escherichia coli*
    • *Staphylococcus epidermidis*
  • Petri dish plates made with TSA
  • Separate bacterial plates prepared in triplicates
  • Plates were incubated at 37°C for 24 hours
  • Kirby Bauer
Methods

• Antimicrobial Tests
  • Minimum Inhibitory Concentration (MIC)
  • Determines the minimum concentration that inhibits bacterial growth
  • 96 well plate tested ZnO and ZrO$_2$ against *E. coli* and *S. epidermidis*
    • Concentrations diluted to 25% and serial diluted in a 1:1 ratio
    • Percent original concentration (25%, 12.5%, 6.25%, 3.125%, ~1.56%, ~0.78%, ~0.39%, ~0.20%, ~0.10%, and ~0.05%).
    • Used a growth control and sterile control
Results and Discussion

• Zinc oxide optical properties from UV-Vis spectroscopy

Figure 1. The UV-Vis absorbance spectrum of zinc oxide nanoparticles from 240 nm to 850 nm.

Figure 2. The first derivative of the zinc oxide nanoparticles’ absorbance spectra with respect to energy dA/dE in electron volts (eV).
Results and Discussion

• Zirconium oxide optical properties from UV-Vis spectroscopy

Figure 3. Zirconium oxide nanoparticles’ UV-Vis absorbance spectrum from 240 nm to 400 nm.

Figure 4. Zirconium oxide nanoparticles’ first derivative of the absorbance spectra with respect to energy dA/dE in electron volts (eV).
Results and Discussion

- Kirby Bauer Test

Table 1. Inhibition zones for ZnO and ZrO₂ nanoparticles against *Escherichia coli* and *Staphylococcus epidermidis*. The ZnO and ZrO₂ NP concentrations were 72.5 mM and 83.6 mM, respectively.

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Escherichia coli</th>
<th>Staphylococcus epidermidis</th>
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<tbody>
<tr>
<td><strong>ZnO</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plate 1</td>
<td>11.0</td>
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</tr>
<tr>
<td>Plate 2</td>
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<tr>
<td>Average ± SD</td>
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<td>Average ± SD</td>
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<tr>
<td><strong>ZrO₂</strong></td>
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</tr>
<tr>
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<td>Plate 1</td>
</tr>
<tr>
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<tr>
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<tr>
<td>Average ± SD</td>
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<td><strong>No Disc Control</strong></td>
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</table>
Results and Discussion

• Minimum Inhibitory Concentration (MIC)

Figure 5. Minimum inhibitory concentration (MIC) Fall 2019 results for ZrO$_2$ and ZnO nanoparticles against *E. coli* and *S. epidermidis*. The initial ZnO and ZrO$_2$ NP concentrations were 72.5 mM and 83.6 mM, respectively.
Results and Discussion

Figure 6. Minimum inhibitory concentration (MIC) Spring 2020 triplicate 1 results for ZrO$_2$ and ZnO nanoparticles against the bacterial strains *Escherichia coli* and *Staphylococcus epidermidis*. Initial ZnO and ZrO$_2$ NP concentrations were 81.3 mM and 68.7 mM, respectively.
Results and Discussion

Figure 7. Average absorbance values from the triplicate minimum inhibitory concentrations (MIC) of ZnO and ZrO\textsubscript{2} nanoparticles against \textit{E. coli} and \textit{S. epidermidis}. Percent concentration refers to the original nanoparticle concentrations, which were 81.3 mM ZnO and 68.7 mM ZrO\textsubscript{2}. 
Conclusion

• The synthesis for both ZnO and ZrO$_2$ are plausible and feasible
  • It would be nice to obtain transmission and scanning electron microscopy of the nanoparticles to further characterize for shape and size

• The ZnO showed some levels of inhibition but not to the same degree as the literature.
  • Improvements could be to try a different synthesis

• ZrO$_2$ nanoparticles showed no signs of inhibition
  • I would be interested to better confirm proper synthesis with TEM and SEM and attempt to test antimicrobial activity again
  • Or it could be that zirconium oxide is not an efficient antimicrobial agent
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References


