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

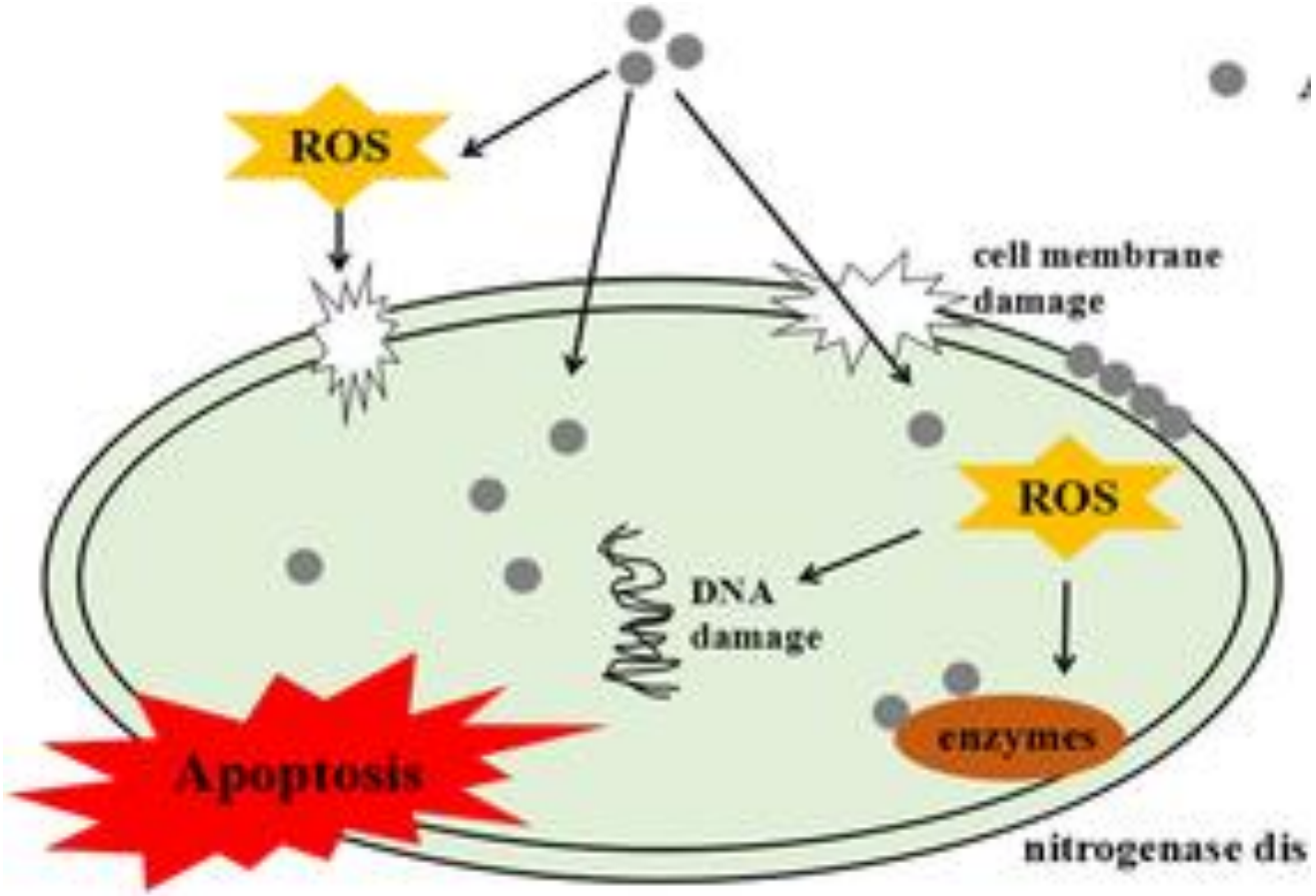
https://crediblemeds.org/blog/antibiotic-resistance-using-antibiotics-intelligently/

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



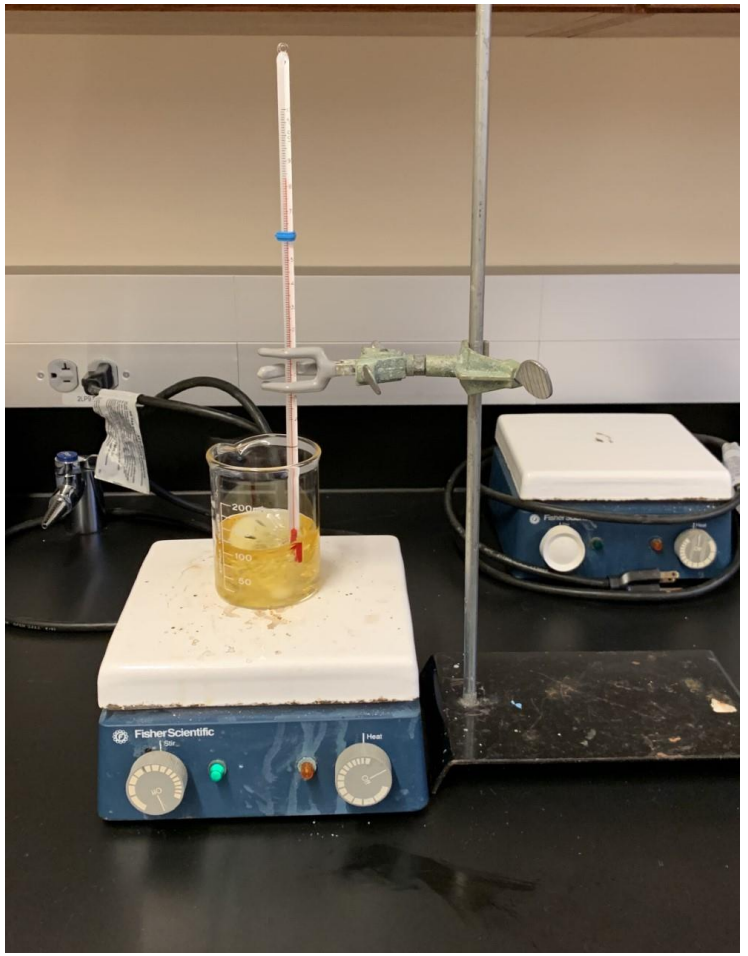
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0209020

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

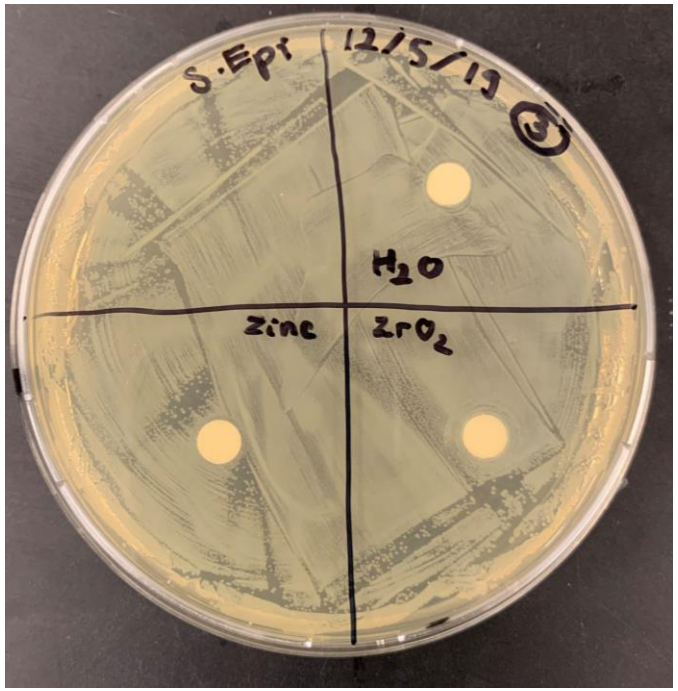
- Zirconium Oxide Synthesis
  - Following Andrea Pucci et al, synthesis occurred under an inert argon atmosphere in the glovebox
  - Benzyl alcohol was purged of oxygen
  - In the glovebox, Zirconium (IV) isopropoxide isopropyl alcohol complex was added with the benzyl alcohol into a Teflon vessel
  - 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

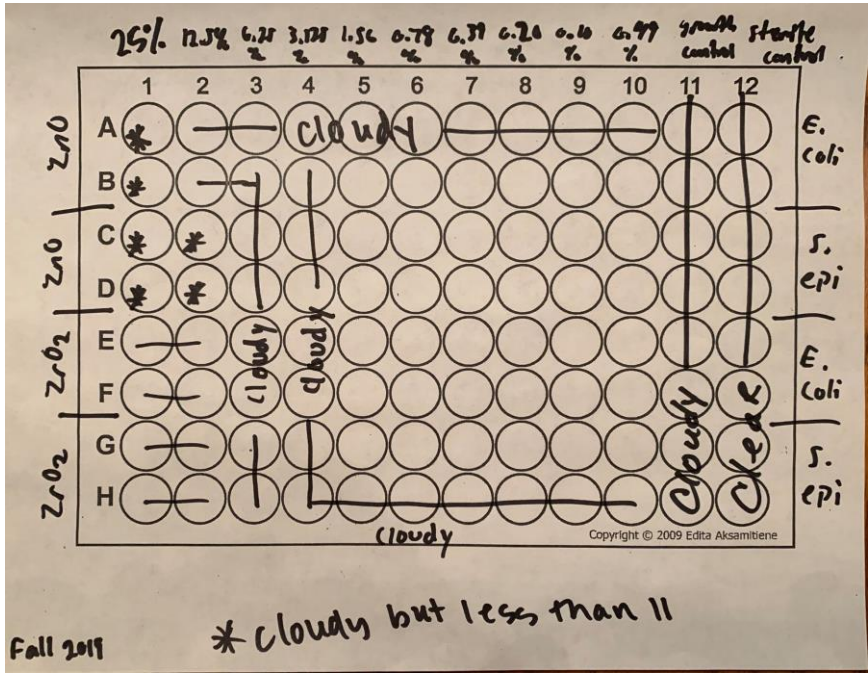
Methods

- 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

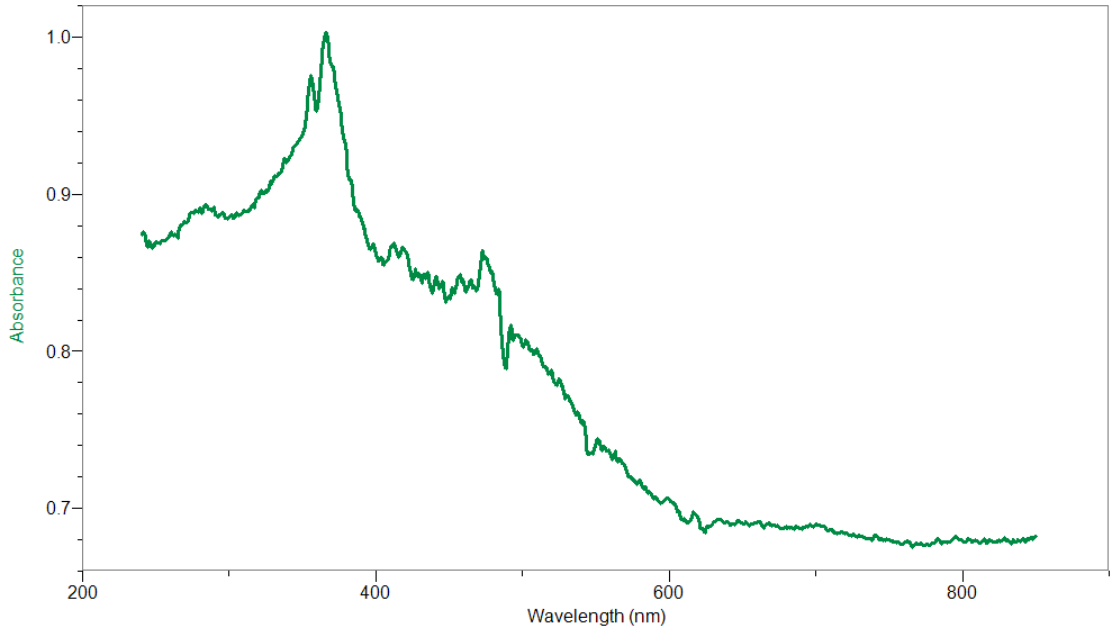


Figure 1. The UV-Vis absorbance spectrum of zinc oxide nanoparticles from 240 nm to 850 nm.

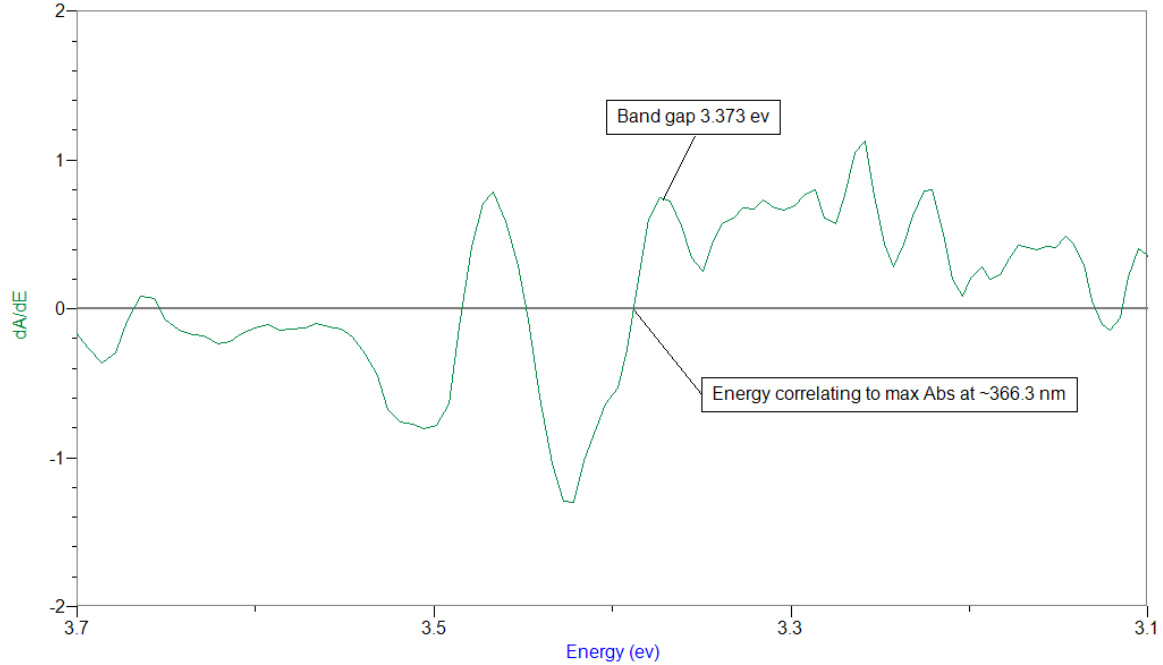


Figure 2. The first derivative of the zinc oxide nanoparticles' absorbance spectra with respect to energy dA/dE in electron volts (eV).

Results and Discussion

- Zirconium oxide optical properties from UV-Vis spectroscopy

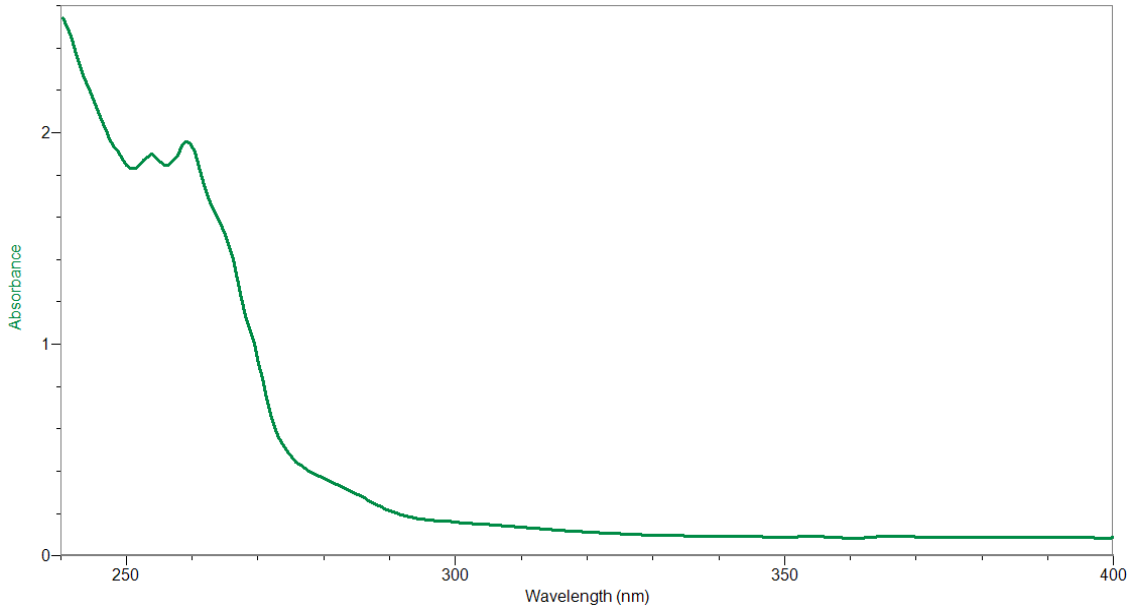


Figure 3. Zirconium oxide nanoparticles' UV-Vis absorbance spectrum from 240 nm to 400 nm.

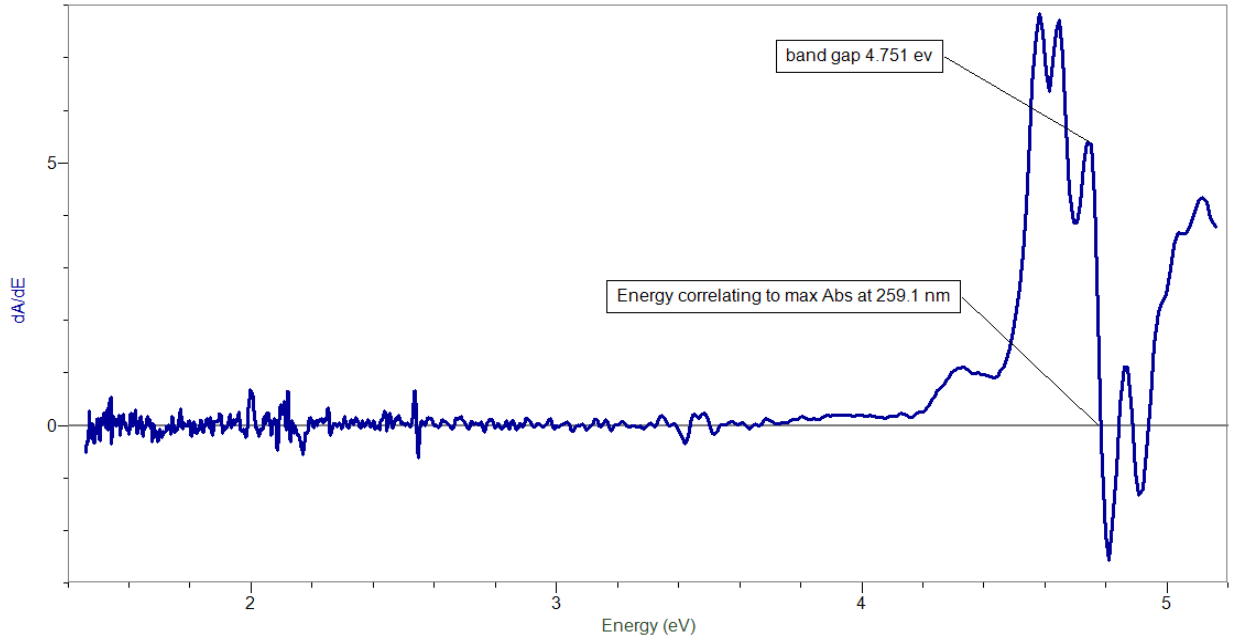


Figure 4. Zirconium oxide nanoparticles' first derivative of the absorbance spectra with respect to energy dA/dE in electron volts (eV).

Results and Discussion

- Kirby Bauer Test

Table 1. Inhibition zones for ZnO and ZrO_2 nanoparticles against *Escherichia coli* and *Staphylococcus epidermidis*. The ZnO and ZrO_2 NP concentrations were 72.5 mM and 83.6 mM, respectively

Zone of Inhibition Diameter (mm)				
Antimicrobial Agent	Escherichia coli		Staphylococcus epidermidis	
ZnO	Plate 1	11.0	Plate 1	17.0
	Plate 2	11.5	Plate 2	13.5
	Plate 3	9.0	Plate 3	0
	Average ± SD	10.5 ± 1.1	Average ± SD	10.2 ± 7.3*
ZrO_2	Plate 1	0	Plate 1	0
	Plate 2	0	Plate 2	0
	Plate 3	0	Plate 3	0
	Average ± SD	0 ± 0	Average ± SD	0 ± 0
MilliQ Water Control	0		0	
No Disc Control	0		0	

Results and Discussion

- Minimum Inhibitory Concentration (MIC)

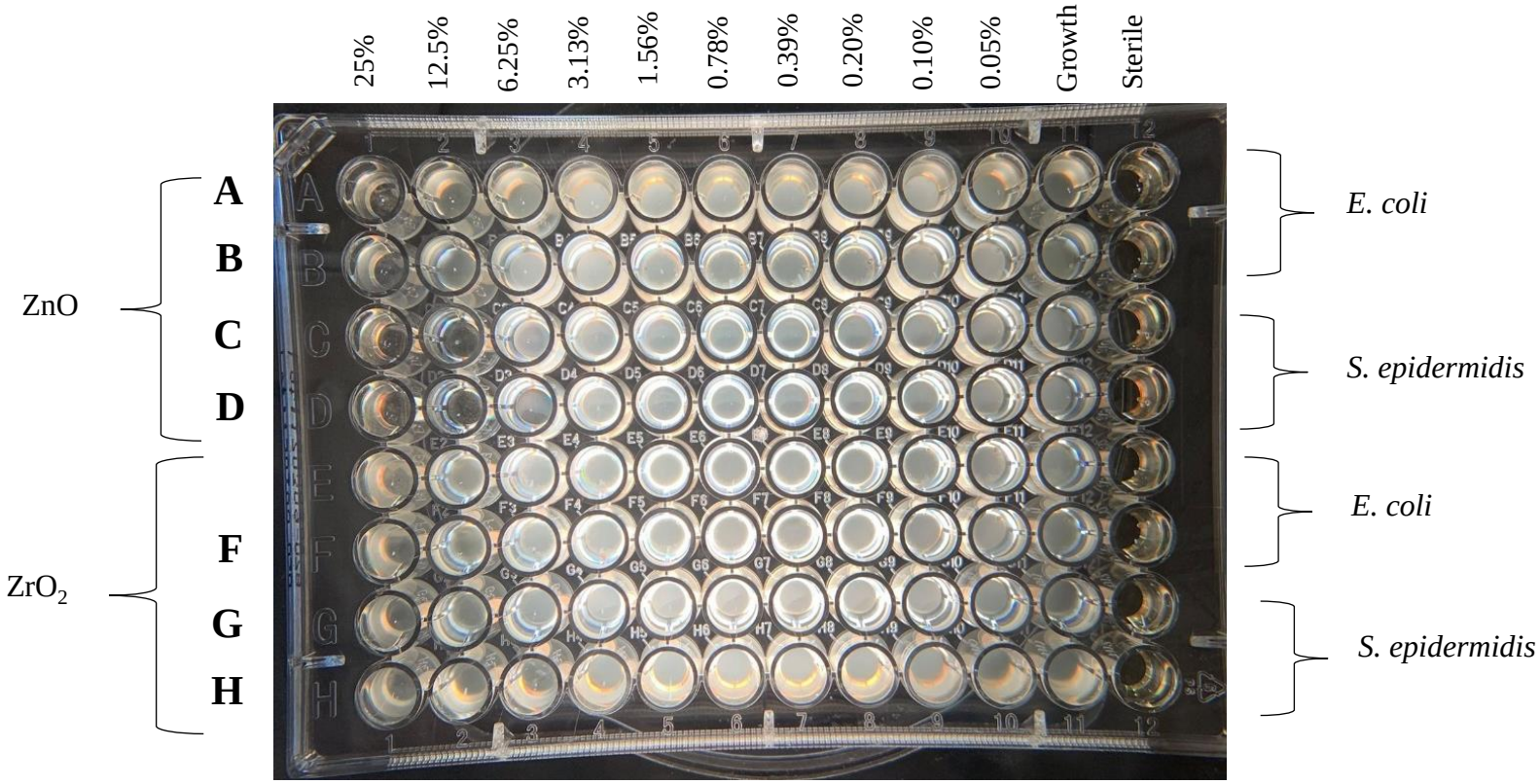


Figure 5. Minimum inhibitory concentration (MIC) Fall 2019 results for ZrO_2 and ZnO nanoparticles against *E. coli* and *S. epidermidis*. The initial ZnO and ZrO_2 NP concentrations were 72.5 mM and 83.6 mM, respectively.

Results and Discussion

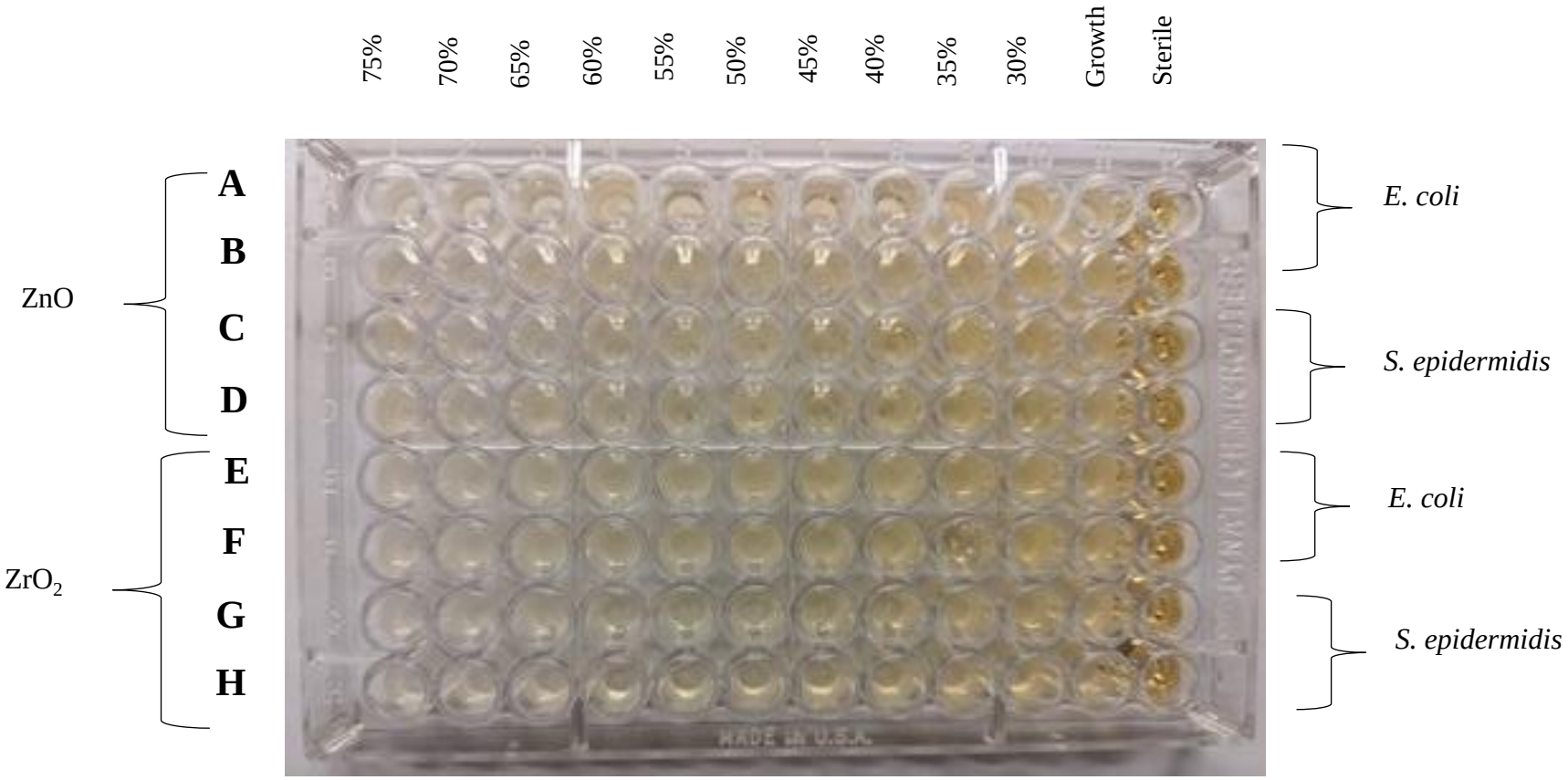


Figure 6. Minimum inhibitory concentration (MIC) Spring 2020 triplicate 1 results for ZrO_2 and ZnO nanoparticles against the bacterial strains *Escherichia coli* and *Staphylococcus epidermidis*. initial ZnO and ZrO_2 NP concentrations were 81.3 mM and 68.7 mM, respectively.

Results and Discussion

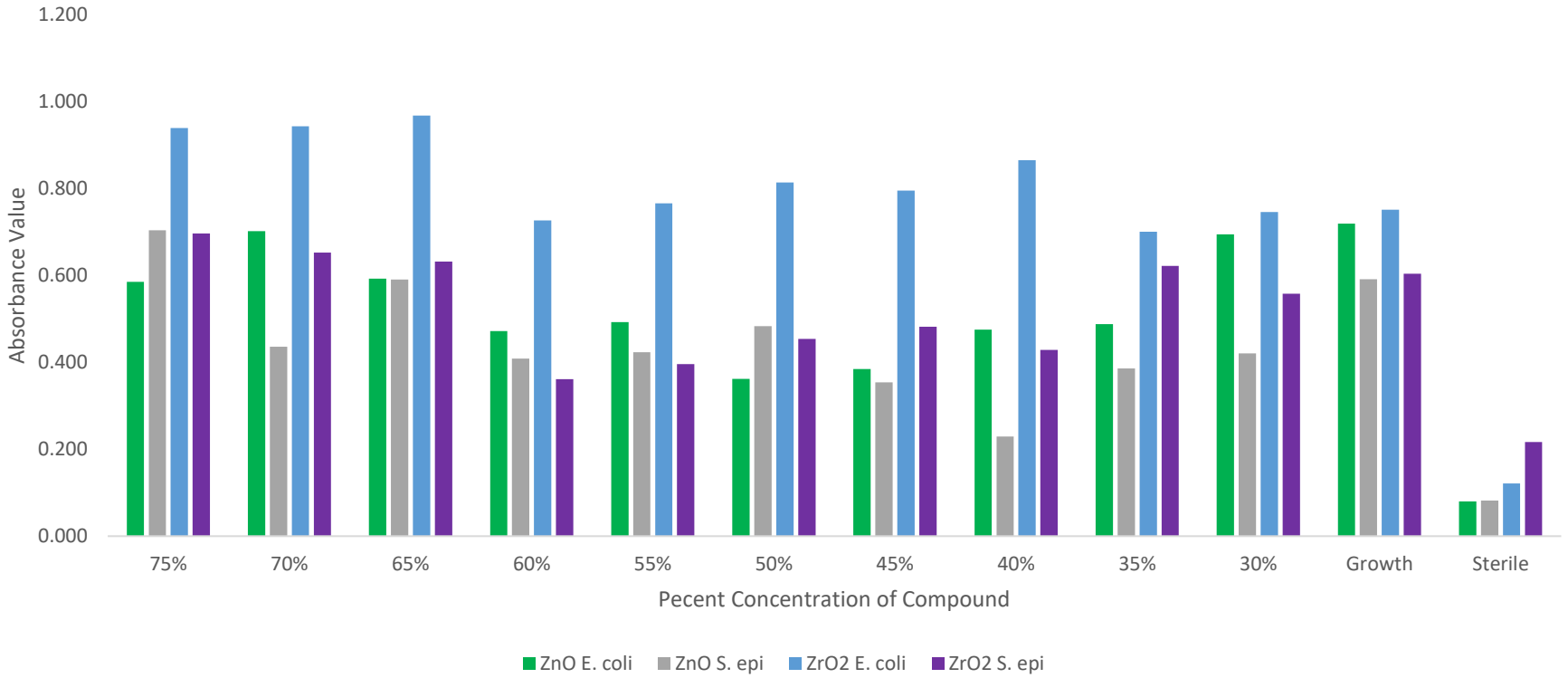


Figure 7. Average absorbance values from the triplicate minimum inhibitory concentrations (MIC) of ZnO and ZrO_2 nanoparticles against *E. coli* and *S. epidermidis*. Percent concentration refers to the original nanoparticle concentrations, which were 81.3 mM ZnO and 68.7 mM ZrO_2.

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

Acknowledgements

- I would like to thank
  - Dr. Gravelle for being my research advisor and helping me through challenges
  - Both Dr. Vohs and Dr. Koehl for their help with the glovebox and bacteria
  - Dr. Hillwig, even though he is no longer here with us, he was my academic advisor for my first 3 years.
  - Dr. Fisher for taking me on as a new academic advisee and for supporting us biochemistry majors for 4 years
  - The entire chemistry department for all you have done these past 4 years. It has been a pleasure and a great learning environment.

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