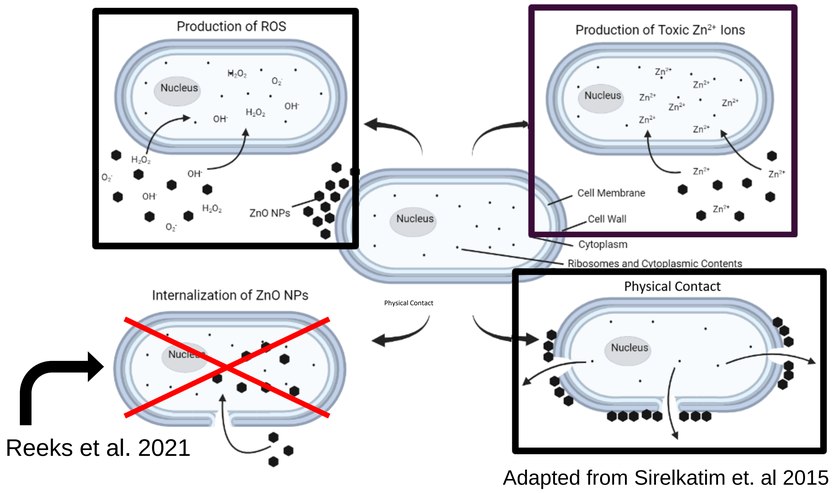
Investigation of the Role of Soluble Species in the Antibacterial Mechanism of Zinc Oxide Against *Staphylococcus aureus*

Alexander Caron, Michael Delgado, Shauna M. McGillivray, Yuri Strzhemechny
Texas Christian University

TCU College of Science & Engineering
Department of Biology

Background

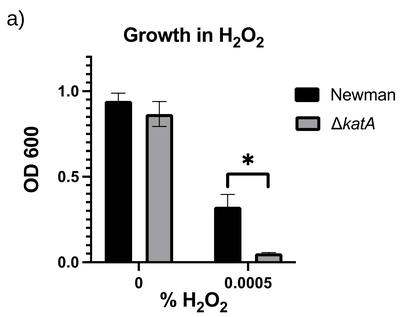
Proposed Mechanisms of ZnO NP Antibacterial Activity

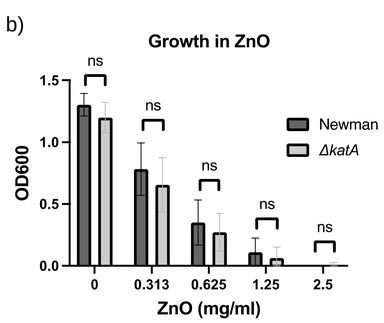


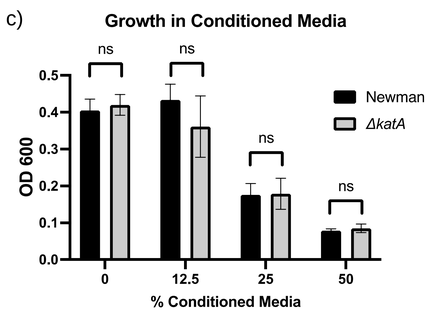
The current antimicrobial action of ZnO is unknown, although several mechanisms have been proposed. Previous work (Reeks et al. 2021) has shown that internalization of ZnO NPs is not necessary for ZnO-mediated killing, consequentially, we chose to investigate the other mechanisms.

H_2O_2 Production Not Critical

$2H_2O_2$ (Hydrogen peroxide) —Catalase→ $2H_2O$ (Water) + O_2 (Oxygen)



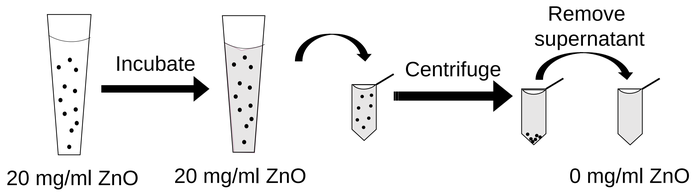




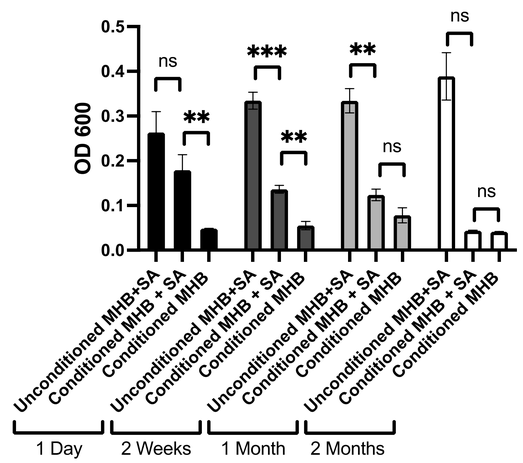
Production of H_2O_2 is not responsible for the antimicrobial activity of ZnO NPs. *p<0.05 by unpaired t-test.

Physical Contact Not Necessary

Method

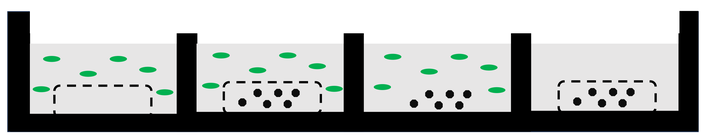


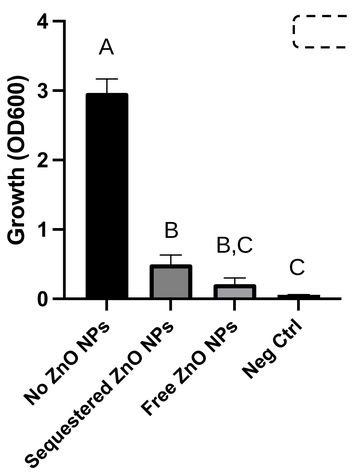
Growth in Conditioned MHB



Conditioned media retains ability to inhibit bacterial growth after removal of NPs. Statistically significant differences represented by **p<0.01, ***p< by one-way ANOVA paired with a Tukey's post hoc test.

Method

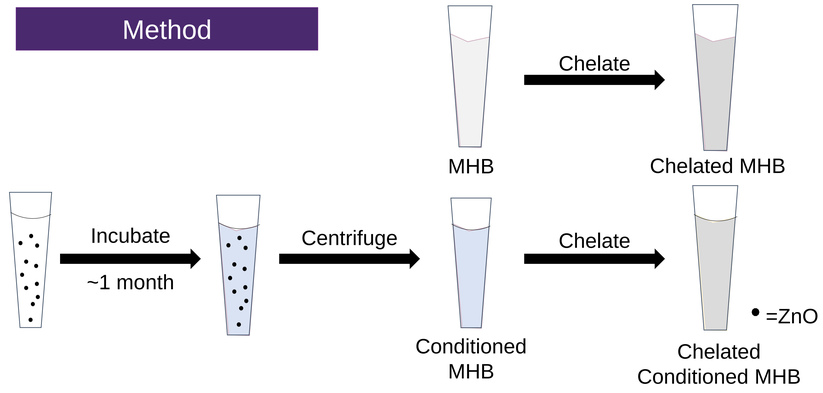




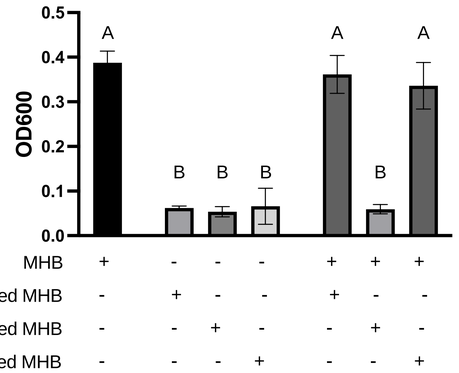
ZnO-mediated growth inhibition is not dependent on physical contact. Statistically significant is indicated by different letters by one-way ANOVA followed by Tukey's *post hoc* test

Zn^{2+} Ions Mediate Toxicity

Method



Growth in Chelated Media



Zn^{2+} concentration in conditioned media

Media Conditions	Zn^{2+} concentration (μM)
MHB	30.8 ± 7.6
MHB conditioned 1 day	3248.9 ± 113.4
MHB conditioned ≥1 month	9588.1 ± 1256
Chelated conditioned MHB	15.9 ± 19.5

Accumulation of Zn^{2+} ions mediate toxicity of conditioned media. Different letters represent statistically significant differences by one-way ANOVA followed by a Tukey's post hoc test.

Conclusions

- H_2O_2 does not mediate ZnO NP toxicity
- Physical contact is not necessary but may still contribute to ZnO toxicity
- Soluble Zn^{2+} is the primary mechanism by which zinc oxide nanoparticles mediate toxicity in Mueller-Hinton Broth

References

John M. Reeks, Iman Ali, William J. Moss, Eric Davis, Shauna M. McGillivray, and Yuri M. Strzhemechny, "Microscale ZnO with controllable crystal morphology as a platform to study antibacterial action on *Staphylococcus aureus*", Biointerphases 16,031003 (2021)

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