Elucidating the mechanisms of CuO ENM bacterial toxicity using time-resolved transcriptional assays

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Introduction and Methods

Better understanding of the mechanisms behind the antimicrobial activity of CuO engineered nanomaterials (ENM) can lead to safer and more efficacious uses of these materials. We used reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) to characterize a time series of *Escherichia coli* gene expression in response to CuO nanoparticle (NP) (100 mg/L as Cu) and equivalent dissolved Cu exposures (1 ppm) added as pulse or gradual inputs.

Results

All treatments elicited a strong, time-dependent induction of Cu-responsive and periplasmic protein damage-responsive gene expression. Both ion treatments led to significantly more induction than NP exposure. Peak responses were typically observed at 30 min for pulse Cu^{2+} exposure and at 60 min for gradual Cu^{2+} and NP exposures. Interestingly, reactive oxygen species (ROS)-responsive genes were not induced for the NP-exposed *E. coli* within the hour time scale where Cu and protein-damage gene expression were most induced – Cu^{2+} led to minimal induction. Only NPs enhanced membrane damage gene expression, which aligns with hyperspectral imaging results that demonstrate a high NP affinity for cellular outer membranes.

Discussion

This study updates our understanding of CuO NP bacterial toxicity and could provide guidance to those applying CuO ENMs, whether looking to exploit their antimicrobial property or hoping to avoid inadvertent impacts. Our data also accentuate factors often neglected in nanotoxicity studies of soluble ENMs. Namely, there is a need both to capture impacts of ENMs at multiple time points and to compare ENM impacts to appropriate ionic controls that reflect the slow ion release from the ENM.