

# Enhancing the antibacterial efficiency of silver-containing magnetic nanocomposites through EDTA surface passivation

Kristin D. Victry, Matthew Corrienti, Cynthia L. Warner, Janine R. Hutchison, Timothy C. Droubay, Ilke Arslan and Marvin G. Warner



Pacific Northwest  
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## Project Overview

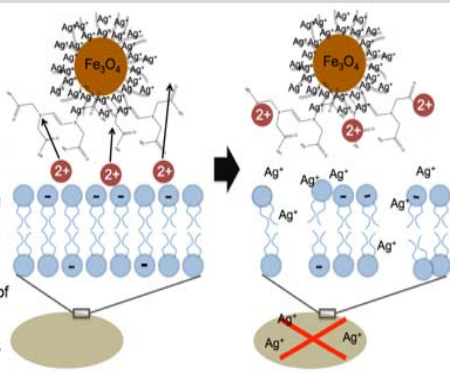
**Objectives:** The purpose of this study is to determine if the antibacterial properties of a magnetic silver nanoparticle composite could be enhanced by the addition of a chelating ligand into the composite. Chelating ligands such as EDTA are known to increase the permeability of the outer membrane of gram-negative bacteria. We propose that the addition of Ag ions, known to be toxic to many bacterial strains, in the presence of EDTA will enhance the antibacterial properties of a magnetic Ag nanocomposite compared to the Ag only nanocomposites.

**Highlights:** Under the looming threat of multidrug resistant (MDR) pathogens, tremendous resources are being devoted to developing silver-based antibacterial technology. The resilience of human cells against silver exposure and the susceptibility of MDR pathogens to even small amounts of silver are primary reasons this element is heralded as the next-generation antibacterial agent.

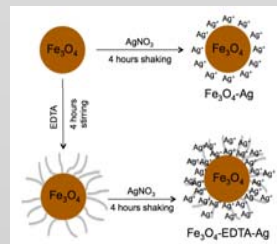
**Method:** This was explored using easy to synthesize EDTA containing magnetic silver nanocomposites for testing against gram-negative *Escherichia coli*, as well as gram-positive *Bacillus subtilis* bacteria strains. Antibacterial efficacy was determined for each composite toward each bacterium using the minimum inhibition concentration, or MIC.

## Mode of Action

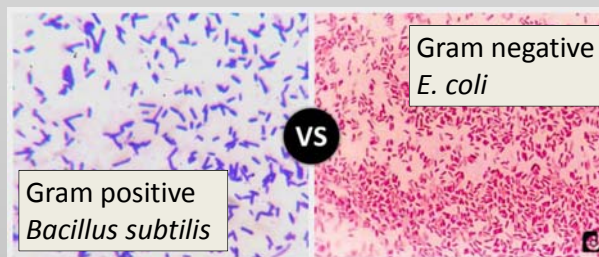
This study describes the effect of including the chelating ligand, EDTA, into a magnetic silver nanocomposite. For gram-negative bacteria, the EDTA chelates the divalent cations present on the outer membrane surface, disrupting the LPS layer and enhancing silver delivery to the cell. The presence of EDTA in the Ag nanocomposite results in a dramatic reduction in the quantity of Ag required to achieve growth inhibition in both gram negative and gram-positive bacterial strains studied.



## Methods



Synthetic strategy for generating magnetic Ag ion and Ag:EDTA coated Fe<sub>3</sub>O<sub>4</sub> nanoparticles



Gram positive  
*Bacillus subtilis*

Gram negative  
*E. coli*

VS



## Minimum Inhibitory Concentration

- 1.5-mL of 10<sup>5</sup>-10<sup>6</sup> CFU/mL
- +  
• geometric dilutions of nanoparticles (12.5-1600 µg/mL)
- 18 hours, 37C, 150 rpm
- technical replicates of 2, 3 biological replicates of each organism

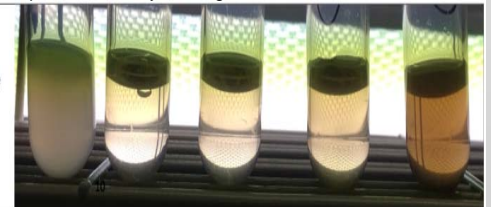
## Minimum Bactericidal Concentration

- 1 mL of each clear culture plated
- 18 hours, 37C

## Results

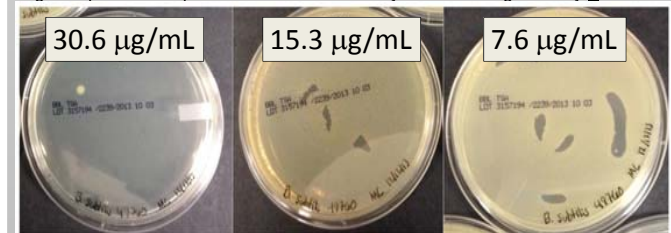
**Minimum Inhibitory Concentration** – lowest concentration of magnetic Ag nanoparticle composite to visually inhibit growth after 18 hours

*B. subtilis* culture inoculated with Fe<sub>3</sub>O<sub>4</sub>-EDTA-Ag



| Ag concentration in solution | 3.8 µg/ml | 7.6 µg/ml | 15.3 µg/ml | 30.6 µg/ml | 61.2 µg/ml |
|------------------------------|-----------|-----------|------------|------------|------------|
|------------------------------|-----------|-----------|------------|------------|------------|

**Minimum Bactericidal Concentration** – lowest concentration of magnetic Ag nanoparticle composite to reduce the viability of the test organism by ≥ 99.9%



**Note:** Non-Ag containing Fe<sub>3</sub>O<sub>4</sub> and Fe<sub>3</sub>O<sub>4</sub>-EDTA showed no growth inhibition at concentrations as high as 1600 µg/mL (data not shown). The minimum inhibitory concentration of non-composited Ag nanoparticles was 25 µg/mL (data not shown).

## Conclusions

The development of bacterial resistance to silver represents a threat to the success of Ag based antibacterial technologies. The Fe<sub>3</sub>O<sub>4</sub>-EDTA-Ag nanocomposite reduces the dosage of Ag necessary to inhibit bacterial growth and is retrievable from liquid environments via magnetic separation, effectively limiting the artificial presence and persistence of Ag in targeted environments.