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Antibacterial effects of silver magnetic nanoparticles against *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and *S.* Anatum

By

Hannah Kruse

An Undergraduate Honors Thesis
submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Bachelor in Science
in Animal and Dairy Sciences
in the Department of Animal and Dairy Sciences

Mississippi State, Mississippi

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Acknowledgements

A line from the movie *Moana* may say it best, “The people you love will change you, the things you have learned will guide you.” In my time at Mississippi State University, I have been blessed with people who have guided me in my education and in my research. I would like to first thank my mother, Dena Kruse, for all of her encouragement in these past few years, and for teaching me the value of hard work and kindness. Thank you to my father, Kyle Kruse, for making me an “interesting person, ” for encouraging me to read hundreds of books, and for ensuring that I received a good education. Thank you to my fiancé, Keven McDowell, for your love and support, and for continuing to inspire me with your intelligence and work ethic. Thank you to my brother, Brady Kruse, for helping me revise my papers and for believing in my research. I would like to thank my New Haven High School teachers and coaches for the lessons that they taught me in the classroom, on the court, and on the track. Thank you to my church family and extended family for your prayers, love, and kindness. I would also like to thank my advisers and instructors at the Department of Animal and Dairy Sciences and in the Shackouls’ Honors College at Mississippi State University for helping me build a solid foundation in my field and in research. Finally, I would like to thank my research adviser, Dr. Jean M. N. Feugang and Dr. Seong Bin Park, not only for their guidance in the laboratory but also for their help in the revision of this thesis. In light of this research, I will leave you with a few thoughts from some other favorite movies of mine:

“Just because something works, doesn’t mean it can’t be improved.” – *Black Panther*

“It’s an imperfect world but it’s the only one we got” – *Iron Man*

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INTRODUCTION

On September 3, 1928, Alexander Fleming noticed an unusual zone of clearing around a blob of *Penicillium notatum* mold in a petri dish (American, 2019). This observation led to the discovery of penicillin and with it began the "antibiotic age" of healthcare (American, 2019). Antibiotics easily cured bacterial infections, saving numerous lives in the process (American, 2019). Not even a century later, healthcare faces a new challenge -- increasing resistance of pathogens to antibiotics. Alternatives to antibiotics must be found, not only because antibiotics are becoming less effective against pathogens in terms of treating infections, but also because excessive antibiotic use has indirectly selected for more resistant, more adaptable pathogens, which have the potential to evolve into superbugs. Silver ions and silver nanoparticles have shown promise in killing or slowing growth of different bacteria and could be used as alternatives to antibiotics. The utilization of silver nanoparticles in wound dressings, surgical masks, and creams has shown its potential in reducing bacterial infections in wounds and its potential in healthcare. Studies have also been conducted to evaluate the use of silver-coated magnetic nanoparticles in reducing bacterial contamination of platelets in stored donated blood (Wang et. al., 2011). Nonetheless, (cyto)toxicities related to the supplementation of biofluids and tissues with silver ions or nanoparticles cause additional concerns.

Meanwhile, the incidence of foodborne illness in United State of America remains a great public health issue. According to the Centers for Disease Control and Prevention (CDC) estimates, 48 million people contract a foodborne illness each year in the United State of America (CDC, 2018). Of these affected individuals, 128,000 are hospitalized and 3,000 die from foodborne illness (CDC, 2018). Five pathogens account for 90% of estimated foodborne illness related deaths. *Salmonella, Listeria, Toxoplasma,* Norwalk-like viruses, *Campylobacter,*

and *Escherichia coli* 0157:H7 (White, et al., 2002). Many of these result in self-limiting diarrheal illnesses of humans, but severe or prolonged illness, especially in immunocompromised individuals, can occur and requires antimicrobial treatment.

Foodborne Pathogens and Antibiotic Resistance

Salmonella enterica

Generalities: *Salmonella* is a rod-shaped, Gram-negative facultative anaerobe in the family Enterobacteriaceae (Eng et al., 2015) Theobald Smith first discovered and isolated *Salmonella* from intestines of pigs infected with swine fever in 1855 (Eng et al., 2015). *Salmonella* is classified into two species, *Salmonella enterica* and *Salmonella bongori*, and this is based on differences in their 16s rRNA sequence (Eng et al., 2015). Nearly all strains are pathogenic (Eng et al., 2015). Strains of *Salmonella* can also be classified based on disease caused in humans as typhoid *Salmonella* and non-typhoid *Salmonella* (Eng et al., 2015). *Salmonella* species are found throughout the environment and can colonize and cause disease in humans and in a variety of animals, and during the initial infection, the bacteria penetrate the epithelial cells lining the intestines and induce phagocytosis-like responses in the cell to gain access into the cell (Eng et al., 2015; White et al., 2002). *Salmonella* can also use this process to survive inside of macrophages and evade the host immune system in this way (Eng et al., 2015). Gastroenteritis is the most common manifestation of *Salmonella* infection, followed by bacteremia, enteric fever (typhoid fever), and chronic carrier state (Eng et al., 2015) Non-typhoidal strains cause a self-limiting gastroenteritis in humans that usually does not warrant antibiotic use (White et al., 2002). Life-threatening systemic *Salmonella* infections do occur and require aggressive treatment with antibiotics and occasionally, chemotherapy (White et al., 2002). Over 2500 serotypes of *Salmonella* have been identified (Eng et al., 2015; White et al. 2002). More than half of these serotypes belong to the *Salmonella enterica* subsp. *Enterica*, which is responsible for most *Salmonella* infections in humans (Eng et al., 2015). Another

serotype known as *Salmonella enterica* serovar Typhimurium is one of the most prevalent serotypes in animals (White et al., 2002). *S.* Typhimurium is associated with gastroenteritis in humans (Eng et al., 2015). It was the most frequently detected serotype in cases of gastroenteritis, as it was found in 29% of clinical isolates in North America (Eng et al., 2015). Ninety-five percent of the 1.4 million annual cases of *Salmonella* infection in humans are attributed to foodborne transmission (White et al., 2002). Contact with farm animals had been implicated in other cases (White et al., 2002). *Salmonella* accounts for 31% of all foodborne illness in the United States (Eng et al., 2015). No vaccine has been developed for gastroenteritis-causing *Salmonella*, and *Salmonella enterica* continues to be one of the most concerning foodborne pathogens (Eng et al., 2015).

Antibiotic Resistance: Antibiotic resistance of *Salmonella* was first reported in the early 1960s, when resistance to chloramphenicol was discovered (Eng et al., 2015). Resistance in *Salmonella enterica* serovar Typhimurium doubled in Great Britain from 1981-1989 (White et al., 2002). Published in 2000, a study conducted from 1985-1987 and then 1995-1998 in Spain revealed that ampicillin resistance in *Salmonella* species had increased from 8-44%; tetracycline resistance from 1% to 42%; and from chloramphenicol from 1.7% to 26% (White et al., 2002). In the U.S., resistance to tetracycline in *Salmonella* increased from 9% in 1980 to 24% in 1990 (White et al., 2002). Use of antibiotics has likely selected for more resistant members of the populations to survive and reproduce (White et al., 2002). Bacteria are also capable of horizontal gene transfer and can easily pass resistance genes to each other without reproducing (White et al., 2002). The number of strains developing Multi-Drug Resistance (MDR) continued to increase and in 2006, 84% of gastroenteritis causing *Salmonella* isolates displayed MDR phenotype (Eng et al., 2015). This is concerning because not only are MDR strains more difficult

to treat with antibiotics, but also MDR Strains are more virulent and cause more severe or prolonged syndromes (Eng et al., 2015). There is also a higher risk of zoonotic transmission of these MDR *Salmonella* (Eng et al., 2015). In 2001, a clinical isolate of *Salmonella enterica* serotype Anatum that was resistant to ceftriaxone was identified in a patient in Chang Gung Memorial Hospital (Su et al., 2003). The patient's infection with *Salmonella enterica* serovar anatum did not respond to the medication of choice, ceftriaxone. According to the case study, "The emergence of resistance to ceftriaxone during therapy for infections caused by *Salmonella* and *E. coli* appears to have been the cause of treatment failure in this patient." (Su et al., 2003). Both serovars of *Salmonella enterica* are developing resistance to antibiotics at an alarming rate, and there is a need for an alternative to antibiotic treatment.

***Escherichia coli* 0157:H7**

Generalities: *Escherichia coli* is a commensal aero-anaerobic Gram-negative bacillus in the family Enterobacteriaceae found commonly in the vertebrate gastrointestinal tract, and some serotypes of *E. coli* can be pathogenic, especially in immunocompromised individuals (Tenaillon, Skurnik, Picard, & Denamur, 2010). Shiga-toxin producing *Escherichia coli* were first recognized as an emerging human pathogen in 1982, when *E. coli* 0157:H7 was implicated in two outbreaks of hemorrhagic colitis (White et al., 2002). Shiga-toxins are lethal cytotoxins produced by some bacteria, and they are released in greater quantities when bacteria lyse (White et al., 2002). Because of this, only certain antibiotics, such as fosfomycin, may be used against Shiga-toxin producing *E. coli* (White et al., 2002). Since 1982, over 200 serotypes of Shiga-toxin producing *E. coli* have been isolated, and 60 of these serotypes have been associated with human disease (White et al., 2002). *E. coli* 0157:H7 has been the cause of large outbreaks

of foodborne illness in North America, Europe, and Asia, and it remains one of the serotypes of most concern, accounting for 3% of foodborne illness in the United States (White et al., 2002).

Antibiotic Resistance: Early isolates of *E. coli* 1057:H7 were susceptible to many antibiotics, and between 1983 and 1958, CDC testing found that only 2 strains (1%) demonstrated resistance to an antibiotic (White et al., 2002). By 1989, an *E. coli* 0157:H7 strain responsible for a waterborne outbreak in Missouri was resistant to three antibiotics: streptomycin, sulfisoxazole, and tetracycline. Another study conducted on human fecal samples from 1989-1991 found that 13 of 176 strains (7.4%) were resistant to those same three antibiotics (White et al., 2002). A 1998 publication reported that 24% of the strains of *E. coli* isolated from humans, animals, and foods displayed resistance to at least one antibiotic, and 19% were resistant to three or more antibiotics (White et al., 2002). There is now data suggesting that resistance to fosfomycin, the drug of choice for treatment of *E. coli* 0157:H7 in Japan, is increasing (White et al., 2002).

Silver-coated Magnetic Nanoparticles

Silver has long been recognized as a potential antibacterial agent. (Santos et al., 2014). In fact, before the discovery of antibiotics, silver was used for its antiseptic activity, especially in treatment of burns and open wounds (Santos et al., 2014). Silver has proven to be effective in inhibition of the replication of both Gram-negative and Gram-positive bacteria (Franci et al, 2015). While the antibacterial effects of silver nanoparticles have been the subject of numerous studies, the mechanism of action is not yet fully understood (Santos et al., 2014). Numerous theories have been proposed. Many studies have reported damage of cell membranes as one mode of action of silver nanoparticles against bacteria (Santos et al., 2014). One theory is that adherence of silver nanoparticles to the cell wall may cause structural changes to the cell wall that increase membrane permeability, and this could result in uncontrolled transport across the cell membrane (Santos et al., 2014). It is also theorized that silver nanoparticles help to generate reactive oxygen species (ROS) and the subsequent formation of free radicals, which can damage bacteria (Santos et al., 2014). Other theories involve inhibition of DNA replication. Silver cations interact strongly with thiol groups in enzymes involved with DNA replication, and silver also can interact with the phosphorus-containing bases of the DNA (Santos et al., 2014). This interaction could prevent DNA replication and cell division (Santos et al., 2014). Protein synthesis has been shown to be altered by treatment with silver nanoparticles (Franci, et al., 2015; Park, et al., 2018).

Smaller sized nanoparticles seem to be more destructive to the bacterial cell wall (Franci et. al, 2015). This could be due to the simple fact that a reduction in the size of nano-sized particle results in an increase in particle surface area, which allows for more chemical molecules

to attach to the surface and enhanced reactivity and toxic effects (Biazar et al., 2011). Nanoparticles with a smaller diameter and a positive zeta potential tend to have interactions with bacterial cells that result in cell death (Franci et al., 2015). Electrostatic forces develop when nanoparticles with a positive zeta potential encounter bacteria with a negative surface charge (Franci et al., 2015). This can promote a closer attraction and interaction between the nanoparticle and the cell and could result in penetration of the bacterial cell membrane (Franci, et al., 2015). Shape may also be a factor in nanoparticle efficacy against certain types of bacteria. *E. coli* appears to be better inhibited by triangular nanoparticles than by spherical and rod-shaped nanoparticles, although all were fairly effective at inhibiting growth of *E. coli* (Franci et al., 2015).

Magnetic nanocomposites are composed of iron oxide nanoparticles that are covered by a layer of porous silica oxides or other compounds, depending upon the intended use of the nanocomposites (Biazar et al., 2011; Prucek et al., 2011). Magnetic nanoparticles of iron oxides move in response to magnets (Prucek et al., 2011). Because of this, they can be of use as effective drug carriers, and they are currently used as MRI contrast agents (Prucek et al., 2011). They are relatively non-toxic to mammalian cells, are biocompatible, and are inexpensive compared to other nanoparticles (Prucek et al., 2011).

Cytotoxicity of silver nanoparticles and silver-coated magnetic nanoparticles is not fully understood and is the subject of much current study. In a study conducted in 2018, fertilized chicken eggs were injected with PBS (control) or pSWCNT-Ag nanoparticles 12 days post-fertilization (Park et al., 2018). At 20 days post-fertilization, embryos were humanely euthanized and examined to evaluate nanoparticle toxicity (Park et al., 2018). According to the study, "*in ovo* injections of fertilized eggs with PBS (control) or pSWCNT-Ag (62.5 micrograms/mL) on

day 12 post-fertilization did not perturb embryo environment.” (Park et al., 2018). Between the control and the nanoparticle treated embryos, no significant differences in skeletal structures and bone mass and no structural abnormalities observed through X-ray imaging, weight of various internal organs such as brain, hearts, liver, spleen, and bursa of Fabricus were observed (Park et al., 2018). However, in another study using silver nanoparticles in rats, severe increase in spleen size, increased T and B cell counts, and accumulation of silver nanoparticles in spleen, liver, lymph nodes, and other organs was noted (Santos et al., 2014). Another study conducted by Xia et al. and Hussain et al. observed the effect of silver nanoparticle treatment in mice and noted abnormal mitochondria size in the liver cells, along with irregular cell shape in mice that had been administered silver nanoparticles (Santos et al., 2014)(Xia et al., 2006;Hussain et al., 2006). As an antibacterial coating on catheters, nano-silver was an effective antibacterial against Gram-positive and Gram-negative bacteria with no significant accumulation of silver detected in the major organs of test animals in which engineered catheters had been implanted (Franci et al., 2015; Roe et al., 2008). One study noted that concentrations of silver nanoparticles below 10 μg/g could be used for therapeutic purposes, but concentrations above that limit would be cytotoxic to bone tissues (Pauksch et al., 2014). Toxicity depends on the size, coating, and chemical component of nanoparticles, as well as where nanoparticles are administered and the duration of treatment with nanoparticles (Biazar et al., 2011). More study is needed to fully evaluate the cytotoxicity risks associated with silver nanoparticles, silver ions, and silver-coated magnetic nanoparticles.

From this background, it appears that both the resurgence of multidrug resistant bacteria and the likely cytotoxicity of silver ions/nanoparticles are concerning, which prompts exploration of new nanocomposites that are constituted of silver ions coating a magnetic core. This magnetic

core allows for subsequent removal from treated specimens. In this research, the antibacterial potential of silver-coated magnetic nanoparticles (Ag-MNP) on food borne pathogenic bacteria was analyzed.

OBJECTIVES

Many studies have demonstrated the bacteriostatic and bactericidal properties of silver, silver nanoparticles, and magnetic nanoparticles. The objective of this study was to analyze the bacteriostatic and bactericidal potentials of silver-coated magnetic nanocomposites (Ag-MNP) against *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *Salmonella enterica* serovar Anatum that were non-invasively transformed to emit light. Four analytical methods were used: bioluminescence imaging for real-time monitoring of bacterial growth, optical density for classical measurement of bacterial growth, solid culture plating for colony forming surviving bacteria, and Transmission Electron Microscopy (TEM) for ultrastructural evaluation of bacteria and Ag-MNP interactions.

MATERIALS AND METHODS

Production of Silver-coated Magnetic Nanoparticles

Iron oxide or magnetic nanoparticles were synthesized by Clemente Associates (Madison, CT, USA), following an unknown proprietary protocol. Coating with dextran further allowed for the attachment of silver ions to form a stable silver-coated magnetic nanocomposites (Ag-MNP). Stock solution (1 mg/ml = ~ 2.5 x 10^6 particles/mg) containing 0.02% sodium azide was stored at 4°C during experiments.

Production of Bioluminescent Bacteria

Strains of *Escherichia coli* O157:H7 (ATTC 43888), *Salmonella enterica* serovar Typhimurium (ATCC 14028), and *Salmonella enterica* serovar Anatum (ATCC9270) were obtained. Each bacterium was cultured in Luria-Bertani broth (LB broth) with shaking in solution and also on solid LB agar at 37 °C. Electroporation of pXen5-*lux*CDABE (Caliper life sciences, Hopkinton, MA, USA) was used to create bioluminescent bacteria that contained a plasmid with genes for production of the luciferase enzyme and luciferin substrate, as well as an ampicillin resistant gene. Successful bioluminescent colonies were selected for on solid agar medium containing ampicillin (100 µg/mL). These cultures were utilized in each study.

Bacterial Growth Analysis with Bioluminescence Imaging

Escherichia coli, *Salmonella enterica* serovar Typhimurium and *S.* Anatum were each grown in LB broth until early stationary phase, and then cell concentrations were adjusted to 1 X 10^7 CFU/mL. Bacteria were incubated for 24 hours with various concentrations of Ag-MNPs (0 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 75 µg/mL, 100 µg/mL, and 200 µg/mL).

Triplicate cultures of each bacteria/concentration pair were prepared and 96-well culture plates were incubated in an In Vivo Imaging System chamber (IVIS, Lumina XRMS Series III system, Perkin Elmer, Waltham, MA, USA) set at 37°C (Figure 1), for bacterial growth imaging. The bioluminescence emission signal was captured every hour for 24 hours to monitor real-time bacterial growth kinetics (Figure 2). The Ag-MNP nanoparticles were separated from the mixtures with a magnet so that the supernatant containing the bacterial cell cultures could be separated (Figure 3). Collected cells were transferred to 96-well plates for CFU evaluations.

Figure 1: In Vivo Imaging System:
IVIS, Lumina XRMS Series III system, Perkin Elmer, Waltham, MA, USA

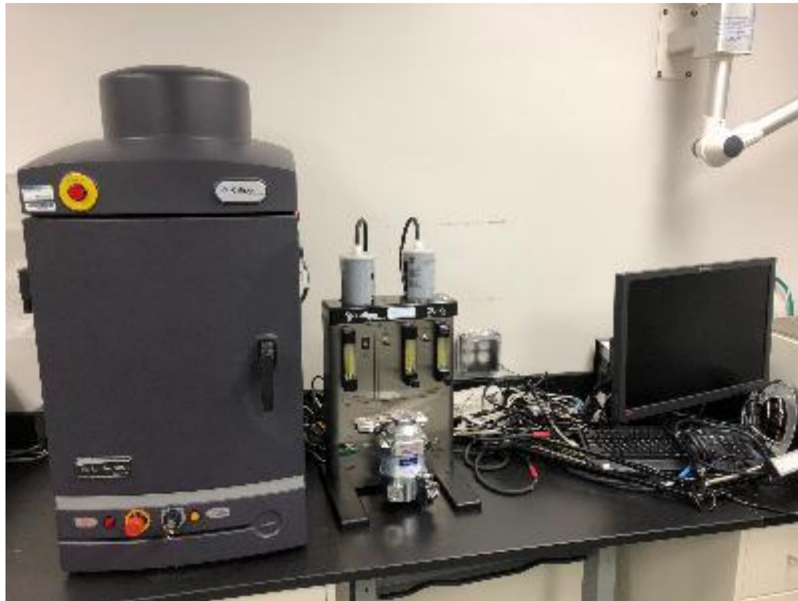


Figure 2: Bioluminescence Imaging with In Vivo Imaging System
(Top Image) Triplicate wells for each bacteria/concentration of nanoparticles combination were used. Nanoparticle concentrations decreased down the plate. Top Row: 200 µg/mL. Bottom Row: 0 µg/mL. (Bottom Image) Growth of bioluminescent bacteria over 24 hours.

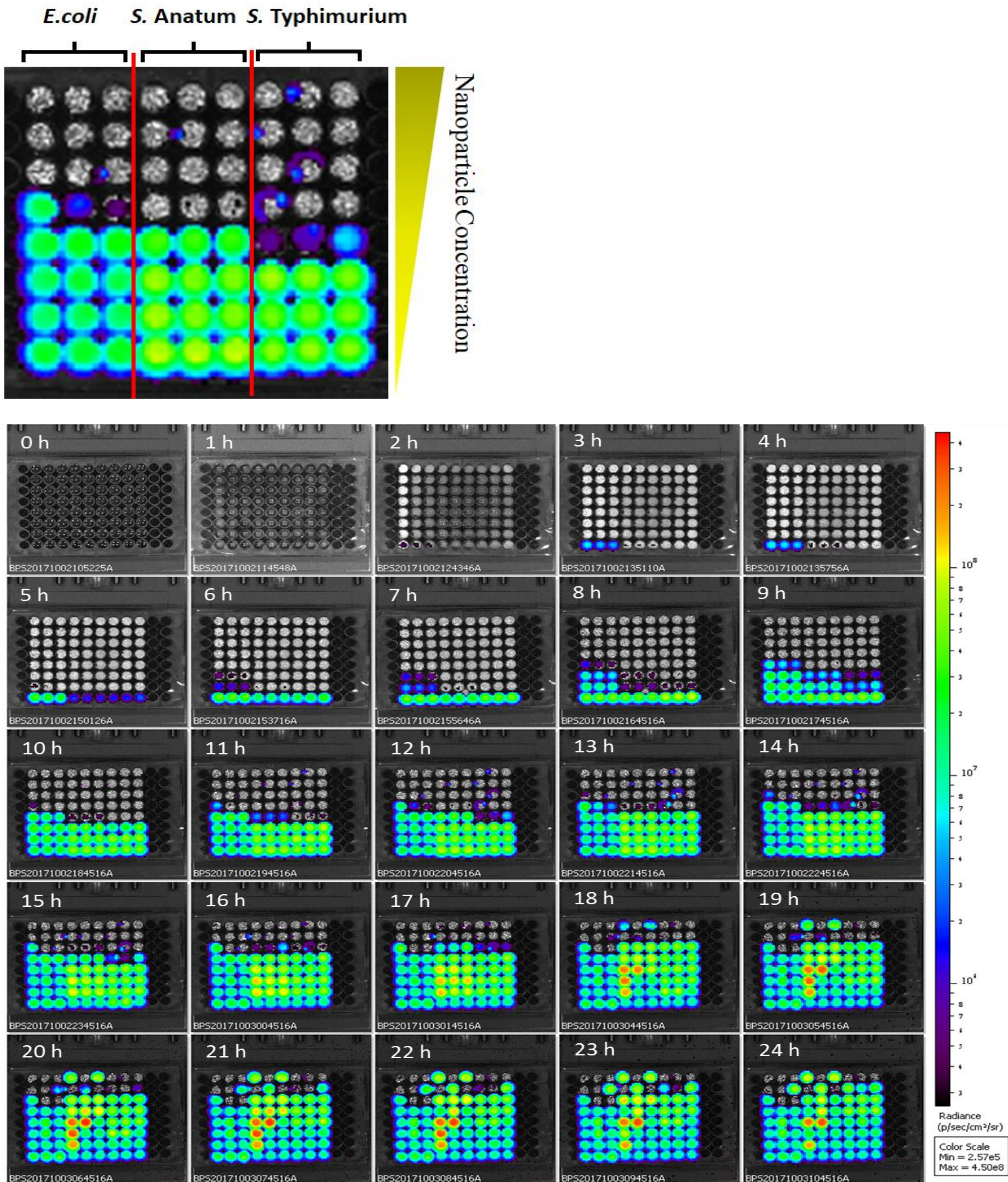
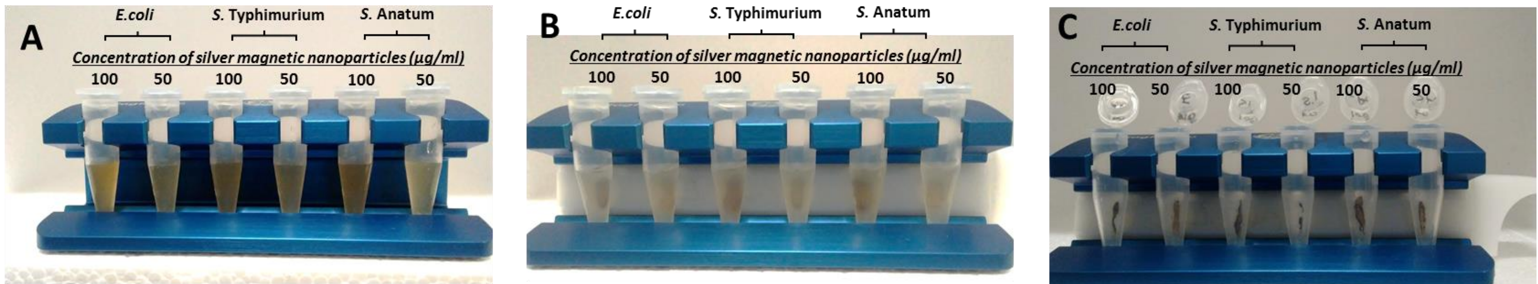


Figure 3: Separation of Magnetic Silver Nanoparticles
(A) Bacteria were lysed after incubation with magnetic silver nanoparticles. (B) Magnetic silver nanoparticles were separated by magnet. (C) Supernatant containing bacterial cells was collected and magnetic silver nanoparticles remained in the tube.



Bacterial Growth Analysis with Optical Density

Escherichia coli, *Salmonella enterica* serovar Typhimurium and *S.* Anatum were each grown in LB broth until early stationary phase, and cell concentrations were adjusted to 1×10^7 CFU/mL. Adjusted cells were incubated for 24 hours with various concentrations of Ag-MNP, from 0 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 75 µg/mL, 100 µg/mL, and 200 µg/mL. Triplicate cultures of each bacteria/concentration pair were prepared, transferred to 96-well plates and incubated for 24 hours in the Cytation 5 Spectrophotometer (Bioteck, Winooski, VT, USA) to monitor bacterial growth at 600 nm optical density (OD). The Ag-MNP nanoparticles were separated from the mixtures with a magnet so that the supernatant containing the bacterial cell could be separated (Figure 3). Collected cells were transferred to 96-well plates for CFU evaluations.

Bacterial Growth Analysis in Solid Phase Culture

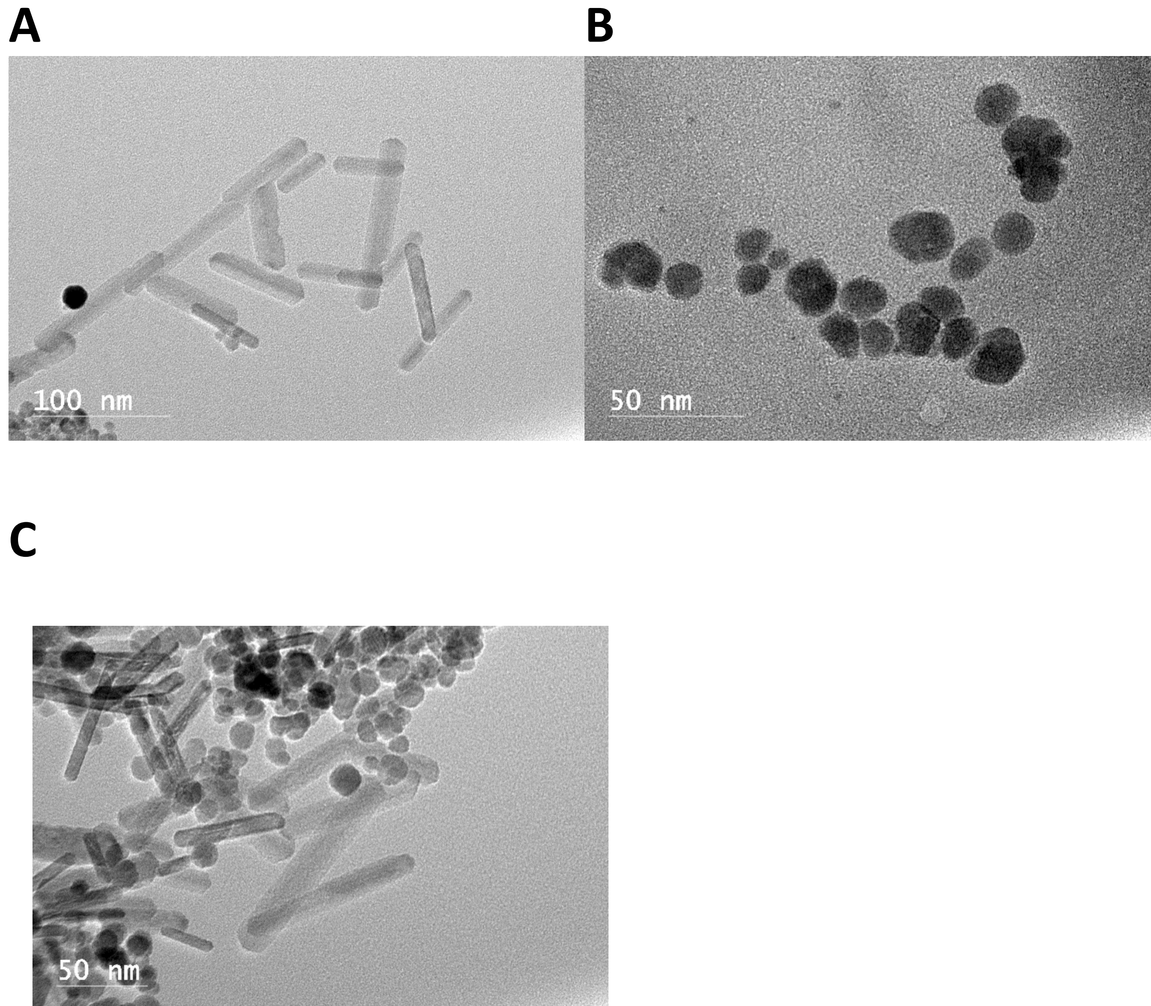
Following incubations of 24 hours in the presence of Ag-MNP (0 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 75 µg/mL, 100 µg/mL, or 200 µg/mL), bacterial growth analyses, and Ag-

MNP removal, aliquots of collected cells above (after bioluminescence and optical density measurements) were cultured at 37 ºC on a solid phase on agar medium with corresponding concentrations of Ag-MNP. The number of live bacteria (CFU/mL) was determined 24 hours later.

TEM Observations

To characterize the synthesized Ag-MNP nanocomposites, the nanoparticles were resuspended in water and subsequently deposited onto a formvar/carbon-coated TEM grid. This was followed by high-resolution transmission electron microscopy imaging (HR-TEM 2100 at 200 kV: JEOL; Peabody, MA, USA) to assess the shape and size of the silver magnetic nanoparticles. The binding of Ag ions to the synthesized iron oxide core was imaged with energy dispersive X-ray spectrometry (EDS: Oxford instruments; Concord, MA, USA). To allow for better understanding of the interactions between silver magnetic nanoparticles and *E. coli*, a sample of *E. coli* at 1 X 10^7 CFU/mL was combined with silver magnetic nanoparticles at 100 μg/mL and co-cultured for 24 hours. Bacteria were fixed with 2.5% glutaraldehyde, centrifuged, re-suspended in distilled water, stained with 1% uranyl acetate, and loaded onto a 100 mesh copper TEM grid. Five independent preparations were performed. Transmission electron microscopy (TEM) was used at 50 nm magnification to view shapes of the mixed nanoparticles and to observe Ag-MNP and bacteria interactions (Figure 4).

Figure 4: TEM Images of Magnetic Silver Nanoparticles. (A) Rod-shaped silver nanoparticle. (B) Spherical silver nanoparticles. (C) Two different shapes of magnetic silver nanoparticles were mixed.



Method of Statistical Analysis

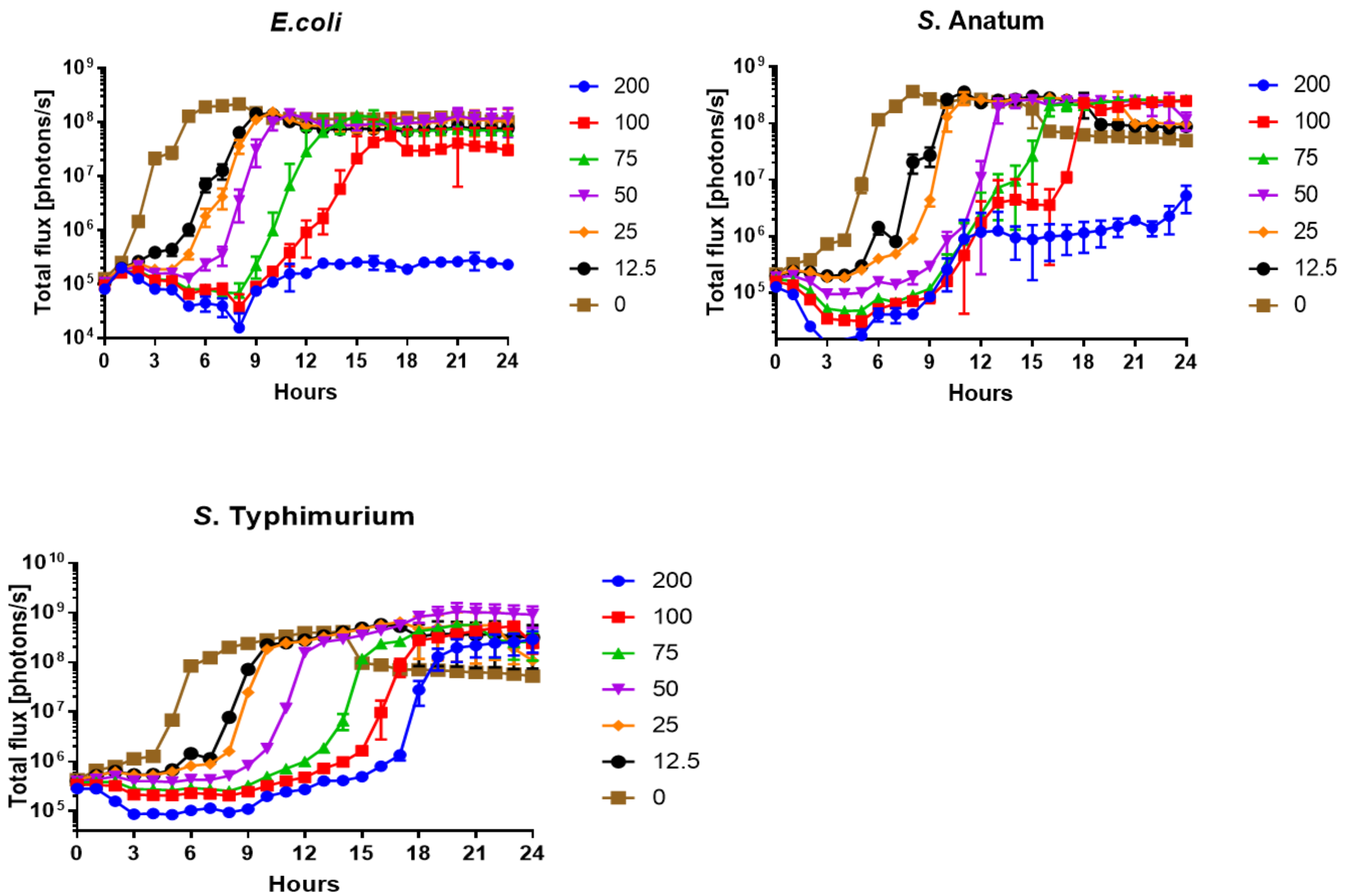
Comparison between nanoparticle treatments of cell cultures were done with ANOVA, followed by a pairwise comparison Student's t-test.

RESULTS

Bacterial Growth Analysis with Bioluminescence Imaging

Bioluminescence imaging showed significant increase in lag time for all bacteria, particularly those incubated with Ag-MNP concentrations of 100 µg/mL and 200 µg/mL (Figure 5). Growth of *E. coli* was nearly inhibited when the bacteria was incubated with 200 µg/mL silver magnetic nanoparticles.

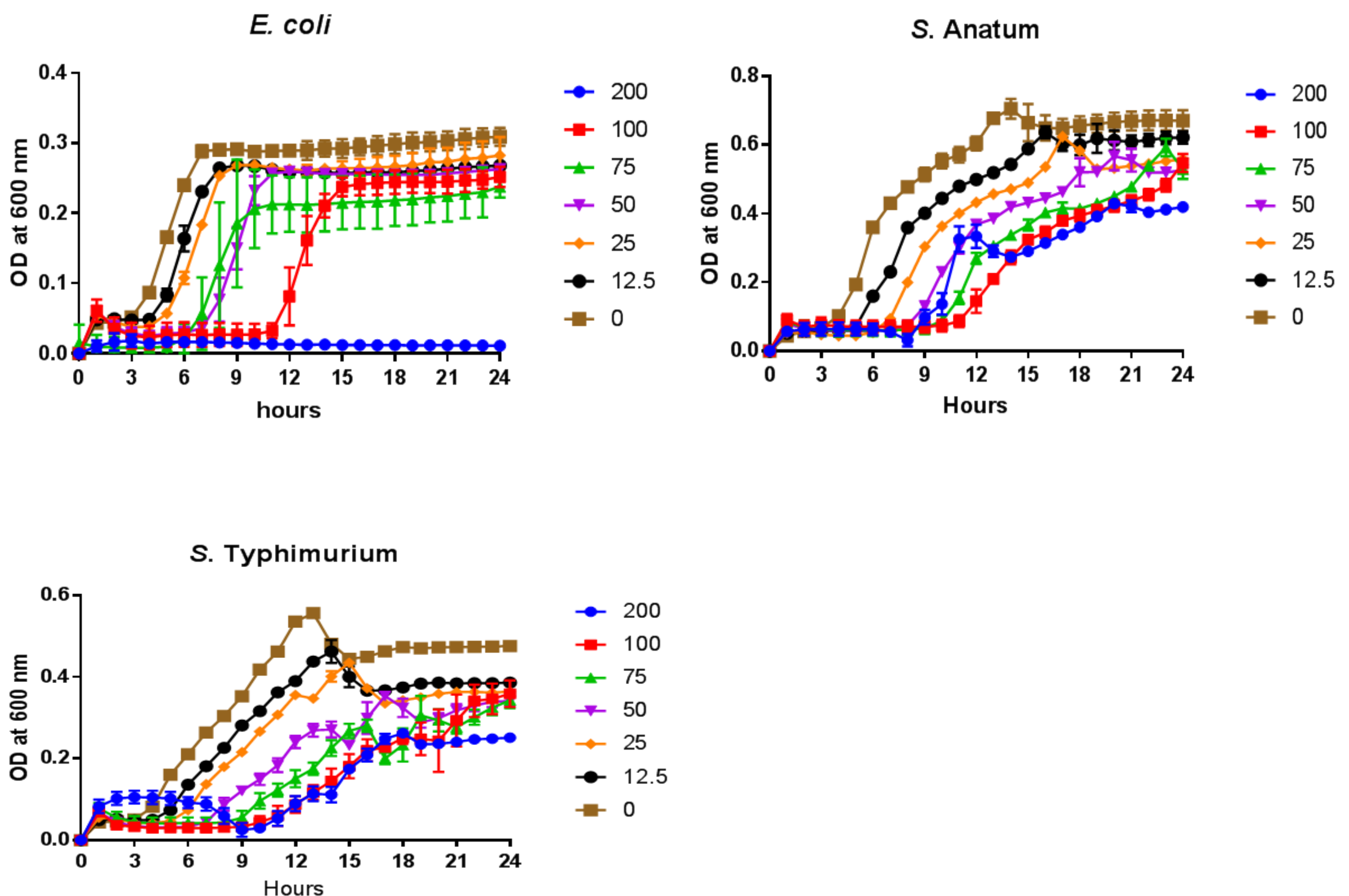
Figure 5: Growth profiles of *E. coli*, *S*. Anatum, and *S*. Typhimurium exposed to various concentration of Ag-MNP - Bioluminescence imaging. Growth curves were monitored by bioluminescent imaging system for 24 h. Points represent the mean with standard deviations from triplicate cultures.



Bacterial Growth Analysis with Optical Density

Optical Density (OD) measurements showed significant increase in lag time for all bacteria. Maximum inhibition of growth of *Salmonella enterica* serovar Typhimurium and *S.* Anatum was 9 hours with nanoparticle concentrations of 100 µg/mL and 200 µg/mL. *E. coli* incubated with 100 µg/mL nanoparticles had lag time of almost 12 hours, and *E. coli* incubated with 200 µg/mL nanoparticles had a lag time of over 24 hours (Figure 6).

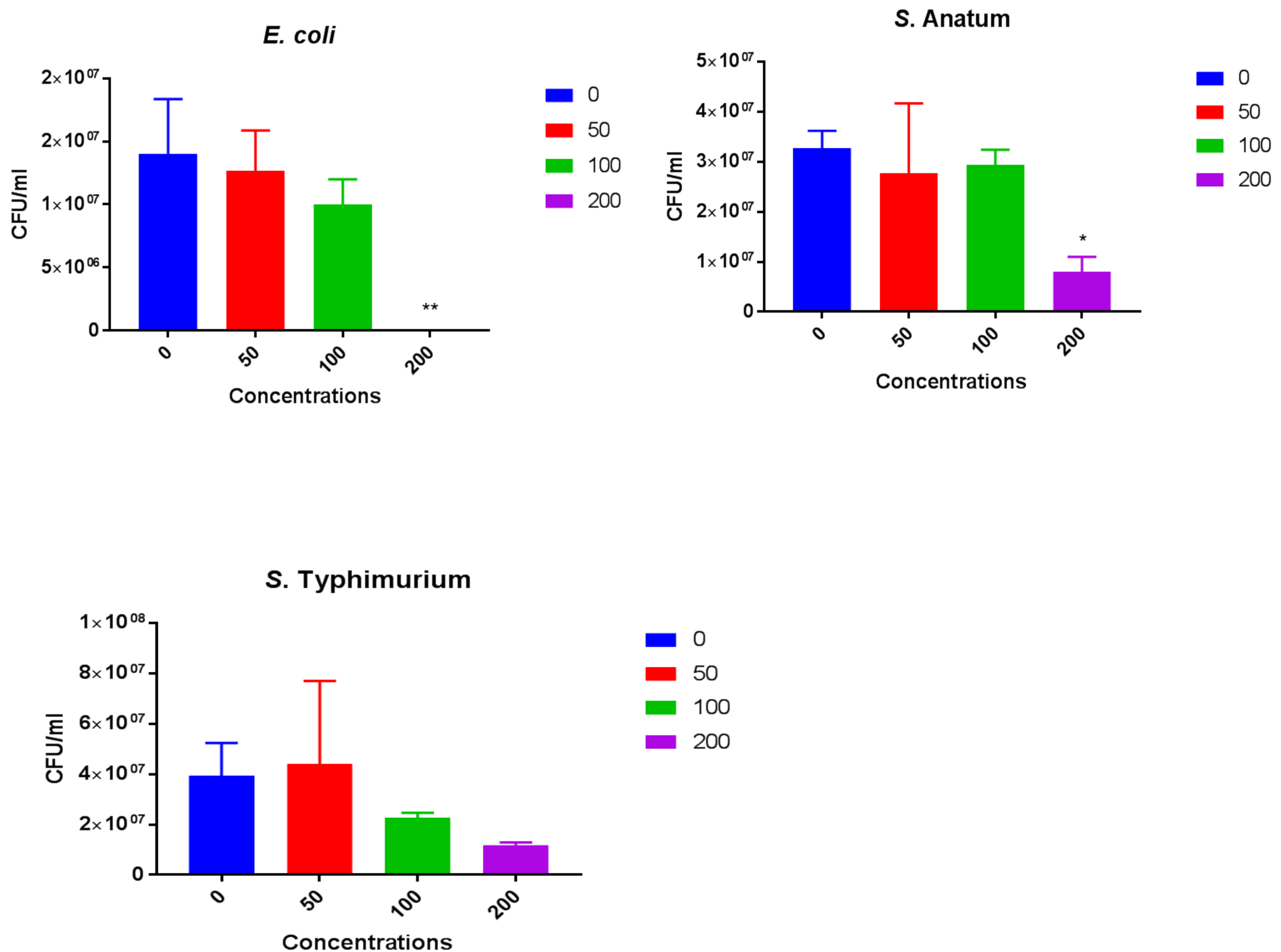
Figure 6: Growth profiles of *E.coli, S*. Anatum and *S*. Typhimurium incubated with various concentrations of Ag-MNP - Optical Density measurement. Optical density was measured at 600 nm for 24 hours. Points represent the mean with standard deviations from triplicate cultures.



Bacterial Growth in Solid Phase Culture

CFU/mL measured at 24 hours after incubation with silver magnetic nanoparticles. CFU counts from culture plates indicated that bacterial growth was increasingly inhibited as silver magnetic nanoparticle concentrations were increased (Figure 7). The CFU data for *E. Coli* showed significant decrease in bacteria concentration in those incubated with nanoparticle concentrations of 100 µg/mL and 200µg/mL(*$P < 0.05$ and **$P < 0.01$).

Figure 7: Survival of bacteria (CFU/ml) of *E. coli*, *S*. Anatum and *S*. Typhimurium exposed to various concentration of Ag-MNP – Colony Forming Units. CFU/ml was measured at 24 hours post incubation with magnetic silver nanoparticles. Data (A) was presented as the mean ± SD of three replicate spots from three independent experiments (*$P < 0.05$ and **$P < 0.01$).

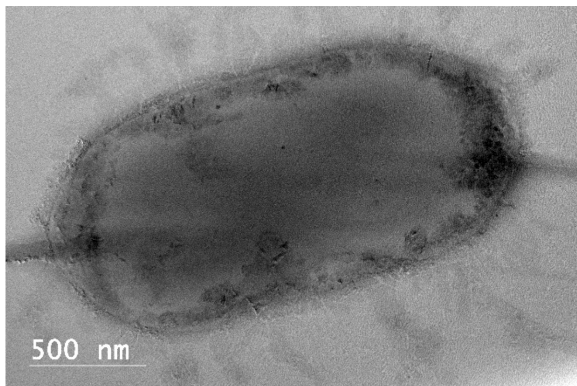


TEM Observation

TEM observation of silver magnetic nanoparticles interacting with the *E. coli* is shown on Figure 8. There were sequential events of Ag-MNP and bacteria interactions, starting with Ag-MNP clustering, attachment and infiltration of the cell membrane, which led to the cell lysis likely due to modifications of the cell structure and function.

Figure 8: TEM imaging of *E. coli* cells. (A) Control *E.coli* with pili and flagella. (B) A cluster of silver nanoparticles was attached to *E.coli*. (C) *E.coli* was surrounded by magnetic silver nanoparticles. (D) *E.coli* showed irregular cell margin with infiltration of magnetic silver nanoparticles.

A



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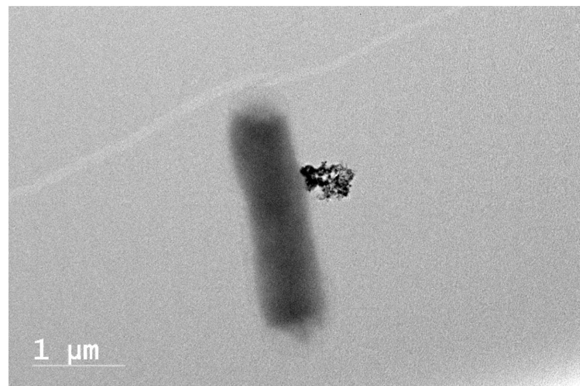

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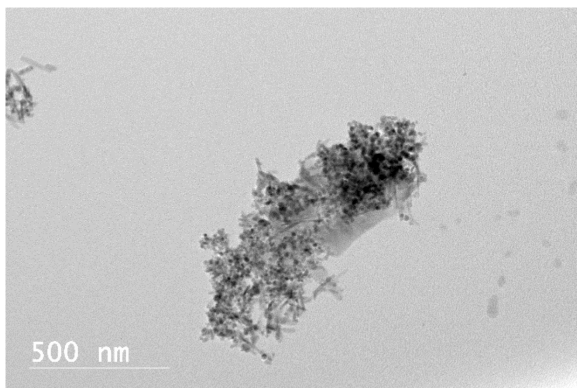

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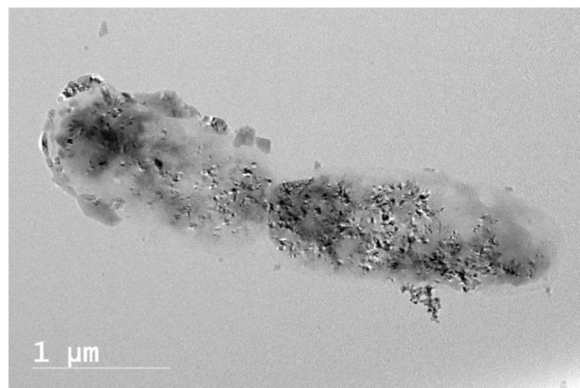

DISCUSSION

In this study, silver-coated magnetic nanoparticles were incubated with *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and *S.* Anatum. After the nanoparticles were removed from the aliquots, bacterial growth over a 24 hour period was measured. From the results of bioluminescence imaging for real-time monitoring of bacterial growth, optical density for classical measurement of bacterial growth, and solid culture plating for colony forming surviving bacteria, it can be concluded that silver-coated magnetic nanoparticles increased lag time of bacterial growth. Increased concentration of nanoparticles resulted in increased lag time, which meant that higher concentrations of nanoparticles were more effective at slowing or preventing bacterial growth. The silver-coated magnetic nanoparticles demonstrated more effective inhibition of growth of *E. coli*, but they were also effective against *Salmonella*. Other studies have also noted this, and some have theorized that silver-coated magnetic nanoparticles are more effective against Gram-negative bacteria, like *E. coli* and *Salmonella*, because of differences in thickness of peptidoglycan in the cell wall compared to that of Gram-positive bacteria (Franci et al., 2015). Transmission Electron Microscopy (TEM) for ultrastructural evaluation of bacteria and Ag-MNP interactions showed nanoparticles successfully attaching to the cell wall of the *E. coli*, surrounding the cell, and infiltrating it. Several of the theorized mechanisms of action of silver-coated magnetic nanoparticles against *E. coli* involve disruption of the cell wall (Franci et al, 2015; Santos et al., 2014). The TEM images demonstrated this phenomenon.

CONCLUSIONS AND FUTURE DIRECTIONS

This study demonstrated the potential of silver-coated magnetic nanoparticles as an antibacterial agent. Maximum inhibition of growth of *Salmonella enterica* serovar Typhimurium and *S.* Anatum was 9 hours with nanoparticle concentrations of 100 µg/mL, and 200 µg/mL. *E. coli* incubated with 100 µg/mL nanoparticles had lag time of almost 12 hours, and *E. coli* incubated with 200 µg/mL nanoparticles had a lag time of over 24 hours. No CFU of *E. coli* were able to grow on solid medium 24 hours after incubation with 200 µg/mL of Ag-MNP. TEM images showed Ag-MNP adhering to the cell membrane of *E. coli* cells and infiltrating the membrane, damaging the cell. Future studies must be done to identify the mechanism of action used by Ag-MNP against bacteria and to evaluate how nanoparticles might be altered to increase efficacy against *Salmonella enterica*. Cytotoxicity of Ag-MNP must also be better evaluated to allow for industry application of Ag-MNP as an antibacterial agent.

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