THE CANADIAN SCIENCE FAIR JOURNAL

ARTICLE

Size Dependency of Magnetic Nanoparticles in Relation to Bacterial Stress Responses

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Canada-Wide Science Fair 2019 Finalist

How does the size of the magnetic nanoparticle utilized in magnetic nanoparticle AMF treatment impact the stress responses and growth of E. coli K12 bacteria? This experiment was performed to test the impact of varying dimensions of magnetic nanoparticles (MNPs) on bacterial growth. These results have the potential to be applied as an alternative antibiotic for antibiotic resistant bacteria if this study can demonstrate that MNPs can induce bacterial apoptosis (bacterial death) through ROS (reactive oxygen species) and thermal stressors (heat stressors). Inspiration for this project came from volunteering at the hospital, and interacting with a patient that had pulmonary multi-drug resistant tuberculosis resulting from HIV immunocompromisation. Witnessing the impacts that a lack of an alternative treatment for MDR-TB had at the hospital, inspired the author to investigate possible methods that could be used to address the issue of antibiotic resistant bacteria.

BACKGROUND

Nanoparticles are defined as solid colloidal particles ranging in size from 10 to 1000 nm, and a type of magnetic nanoparticle (MNP), iron (III) oxide (IONP), displays unique magnetic properties based off of their size (Figure 1) (Martinez, L. 2018). IONPs display superparamagnetism (the absence of a coercivity loop, otherwise known as a magnetization loop) when they reach dimensions of less than 20 nm, and the particles lack magnetic hysteresis (the retention of magnetism) (Goya, G. F., Berquó, T. S., Fonseca, F. C., & Morales, M. P., 2003). This means that the IONPs become magnetized in a magnetic field, but their magnetism disappears when the field is removed (Martinez, L. 2018). This is the opposite of ferromagnetic IONPs, which have larger dimensions (above 20 nm), and retain their magnetic properties when the magnetic field is removed (magnetic hysteresis) (Lima, E. et al., 2013). E. C. Holland claimed that the absence or presence of magnetic hysteresis depends on the quantum effects of spin, or the direction of the momentum of the MNPs (personal communication, Februarys 11, 2019).

Neel-Brownian relaxation is the flip-flip action of MNPs when exposed to an alternating magnetic field. Neel relaxation time is an exponential function of grain volume, which is why the flipping probability decreases the larger the NP becomes (Kötitz, R., Fannin, P., & Trahms, L., 1995). In regards to the impacts that Neel-Brownian relaxation has on its environment, the flipping motion releases thermal energy into its surroundings (Kötitz, R., Weitschies, W., Trahms, L., & Semmler, W., 1999). This thermal energy causes hyperthermia (temperatures between 41-46 degrees Celsius), inducing heat stress responses in bacteria, and the denaturation of proteins (Boor, K. J., 2006). Additionally, Pankhurst and Connolly have described that the friction cause by Neel-Brownian relaxation causes a reaction between the IONPs and free oxygen particles within the cell, resulting in the formation of harmful reactive oxidative species (ROS) (personal communications, December 16, 2018). ROS possess an unpaired electron, which makes them highly reactive, and therefore able to damage all macromolecules, including lipids, proteins and nucleic acids (Boor, K. J., 2006). If MNP therapy is applied to bacterial samples, then heat and oxidative stress responses are activated in the bacterium (Poole, & Keith. 2012). However, while sufficient application of hyperthermia can result in cell death, stressed-induced mutagenesis (when external stressors cause the genes within an organism to mutate to adapt to the stressors) can cause surviving bacterial cells to develop thermotolerance (resistance

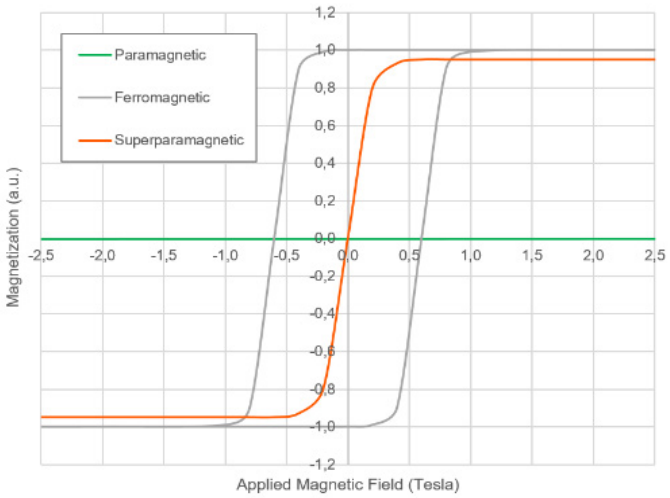

Figure 1. Magnetic coercivity graph of ferromagnetic, paramagnetic, and superparamagnetic nanoparticles.

doi: 10.18192/csfj.v2i32020103106

to heat) and heightened SoxR oxidative stress responses (the protein, SoxR, which responds to ROS by preventing the damaging properties of free oxygen radicals) (Robinson, R. B., & Siegelbaum, S. A., 2003; Poole, & Keith., 2012).

HYPOTHESIS

If bacterial samples are exposed to 10 nm iron oxide nanoparticles, then the bacterial growth in the sample will decrease significantly. This is because of the superparamagnetic properties prevent the adhesion of the nanoparticles, allowing for ready entrance into the bacterium, inducing oxidative stress and hyperthermia) (Martinez, L. 2018; Boor, K. J., 2006).

PROCEDURE

A Luria-Bertani (LB) medium *Escherichia coli* (*E. coli*) K12 bacterial plate with a dilution factor of 106 was created, and after 12 hours, sterile pipette tips were used to select a single colony from the agar plate. The tip was dropped and swirled in the 15 mL glass test tube, which contained 10 mL liquid LB and 2 mL 10 nm IONP liquid medium. The optical density (OD) was tested through pouring 0.8 mL of the sample into a cuvette, and measuring the OD using a spectrophotometer. The culture was loosely covered with sterile cap, and the bacterial culture was incubated in a shaking incubator at 37°C for 12 hours. This procedure was repeated for the 50 nm, 100 nm, and controlled samples. The OD of the samples was recorded every 2 hours.

After 12 hours, the OD of the 10 nm IONP sample was recorded. The sample was then placed between two rotating electromagnets for 5 minutes, with an Arduino Thermal Sensor recording the temperature change per second. After exposing the 10 nm IONP sample to the alternating magnetic field (AMF), a ROS (reactive oxygen species) live cell detection assay was conducted using red fluorescence by staining the 0.8 mL of the sample cells with 0.2 mL of ROS Red Working Solution and a fluorometric microplate reader. The whole sample was incubated for another 2 hours. The procedure of exposing the sample to the samples to the AMF, then measuring the ROS concentration and OD, was continued for another 6 cycles at 2 hour intervals, or another 12 hours. The same was done for the 50 nm, 100 nm, and controlled samples.

After 12 hours, a neodymium magnet was used 5 cm above the samples to extract any excess IONP solution; then the volume was measured. After the experimentation was over, all liquid and solid wastes were safely disposed of, and ethanol was used to disinfect test tubes and petri dishes. This experiment was repeated for another 3 trials.

RESULTS

Summary

The results of this experiment depict the bacterial viability (Figure 2), IONP absorption rate (Figure 3), ROS concentration (Figure 4), and temperature change of each of the samples (Figure 5). The viable bacterial dynamic demonstrated that the three samples had similar patterns of growth before exposure to the AMF. After exposure at 12 hours, the 10 nm IONP samples had the highest

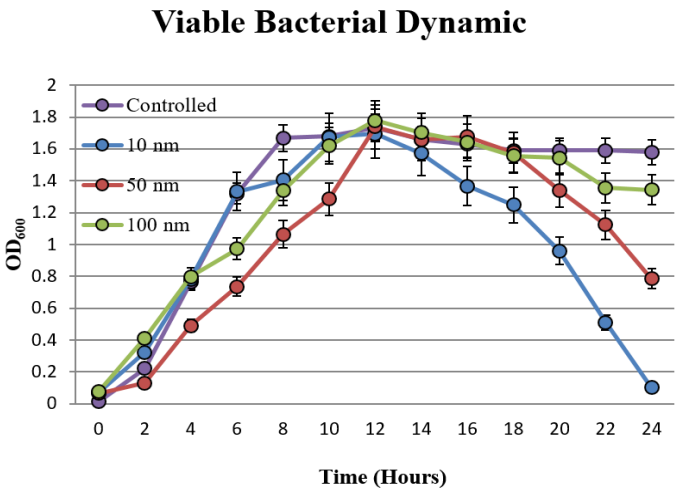

Figure 2. Optical density of bacterial samples over 24 hours.

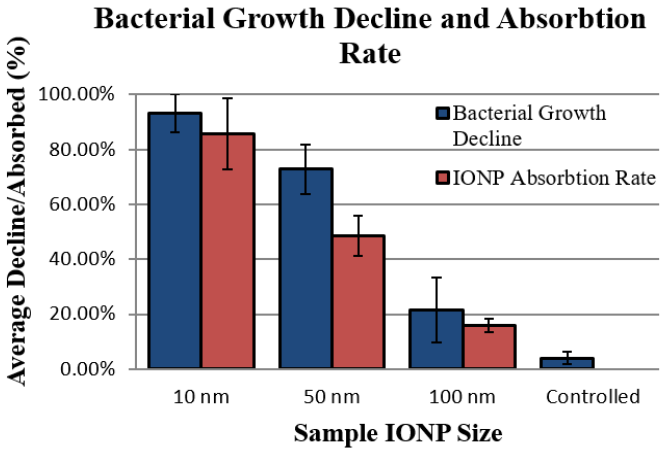

Figure 3. Average IONPs absorbed by samples.

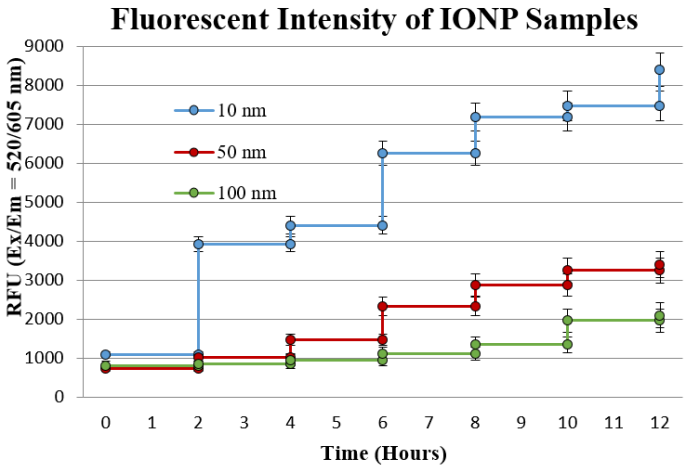

Figure 4. Number of ROS in samples measured by relative fluorescence.

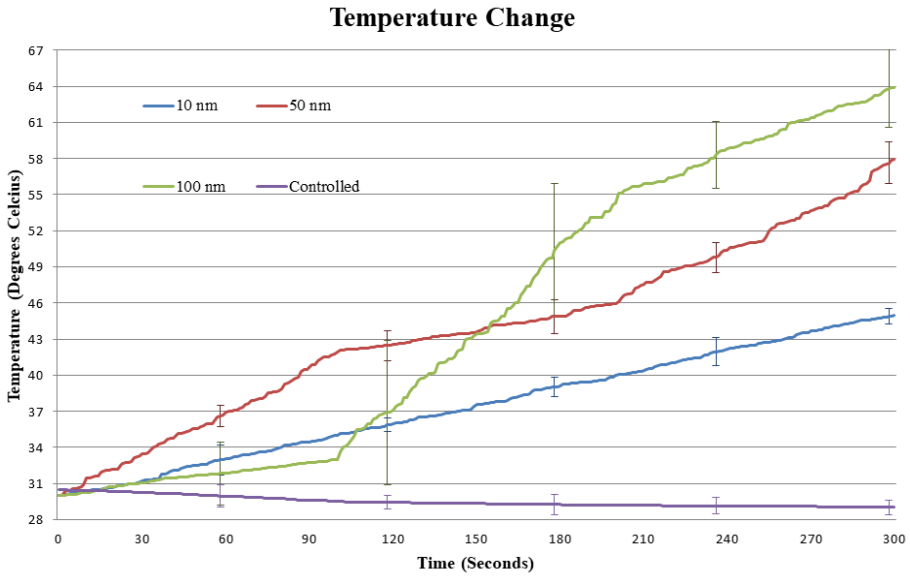

Figure 5. Average temperature change during second intervals.

bacterial decline, the 50 nm IONP samples had an approximate decline that was in between the 10 nm and 100 nm IONP samples, and the controlled and 100 nm samples had similar results of little to no decline. The IONP absorption rates show the percentage of IONPs that the bacteria absorbed, with the 10 nm samples having the highest absorption, 50 nm samples having the approximate average, and 100 nm samples having the lowest. The ROS concentration of the samples shows that the 10 nm sample had the highest ROS concentration, with the 50 nm and 100 nm samples having similar ROS concentrations. The temperature change depicted two observations: patterns of temperature change and duration of the samples in hyperthermic temperature range. The 100 nm samples depicted high deviations from its median temperature, while the 50 nm and 10 nm samples having lower deviations to their median temperatures. The 10 nm samples reached hyperthermia temperatures of 41 degrees Celsius at four to five minutes, 50 nm samples at one to two minutes, and the 100 nm samples at two minutes.

DISCUSSION

The results of the experiment support the project's hypothesis, which states that the bacterial growth in the 10 nm IONP samples will decrease significantly in relation to the controlled, 50 nm, and 100 nm IONP samples. This decrease may have been caused by the high concentration of ROS, and optimum hyperthermia exposure, ultimately inducing bacterial apoptosis. The 10 nm IONP samples were successful due to its superparamagnetic properties and the size-to-volume ratio of the IONPs. The superparamagnetic properties lead to a linear increase in temperature when exposed to AMF while staying within the hyperthermia threshold of 41-46 degrees Celsius. This prevented stressed-induced mutagenesis, avoiding antimicrobial resistance of HSR proteins (heat shock response proteins) to temperatures above the hyperthermia threshold (Poole, & Keith., 2012). This is due to the consistent Neel-Brownian relaxation that is present in superparamagnetic iron oxide nanoparticles (SPIONS) combined with the lower specific power loss (SPL) that is a property of superparamagnetic materials, which causes more consistent flip intervals and a lower amount of thermal energy released (Lima, E. et al, 2013).

The temperatures that ferromagnetic IONPs like the 50 nm and 100 nm samples reached was beyond the hyperthermic limit, which may have caused the surviving bacterial cells to develop thermotolerance, as past studies have established that higher temperature exposure will result in this phenomenon (Poole, & Keith., 2012). Also, the high surface area-to-volume ratio combined with the high absorption rates of the SPIONs (due to its small size) allows for increased exposure of the SPIONs to oxygen particles within the cell, creating ROS (Lima, E. et al, 2013). The lower rates of IONP absorption can be seen in the 50 nm and 100 nm IONP samples. The 100 nm IONP samples were observed to not be able to enter into the cell due to the small surface-area-to-volume ratio and high molecular weight.

These results can be applied in medical treatments for antibiotic resistant strains of bacteria. An example of this would be multi-drug resistant M. Tuberculosis (MDR-TB), as treatment with IONPs would avoid introducing the bacteria to higher levels of antibiotics, thus preventing extensively-drug resistant M. Tuberculosis to occur (Dooley, S. W., Jarvis, W. R., Marione, W. J., & Snider, D. E., 1992). IONPs hyperthermic, oxidative, and delivery capabilities, allow for an indeterminate style treatment and flexibility of the treatment plan, allowing it to be readily accessible for the treatment for the half a million people worldwide that are affected by this fatal disease (Dooley, S. W., Jarvis, W. R., Marione, W. J., & Snider, D. E., 1992). SPIONs are an attractive option for such a treatment, because of their superparamagnetic properties combined with their physiochemical properties (Goya, G. F., Berquó, T. S., Fonseca, F. C., & Morales, M. P., 2003). One of the physiochemical properties of IONPs are its reactivity, as IONPs are known to react with free oxygen particles to create ROS, as stated in a study done by Pankhurst and Connolly (personal communications, December 16, 2018). The physicochemical properties of IONPs also allow for the multi-functionalization of the NPs, allowing for specific issues such as toxicity and other targeted systems such as drug delivery to be implemented through an external coating (Arias, L. S. et al, 2018). As mentioned before, toxicity is an issue that IONPs pose, as past studies one by Pankhurst and Connolly have shown that if the IONPs are deliv-

ered intravenously, they tend to clump in low pressure areas within the vessels, which cause friction between the IONPs and the vessel, releasing ROS and damaging surrounding cells (personal communications, December 16, 2019).

Future directions for this project would be in formulating multi-functionalized NP (MFNP) to treat MDR-TB through lowering the associated toxicities, and making the NP more biocompatible, to allow for interaction with surrounding normal cells, while killing the TB bacteria. This would be accomplished through exploration of possible external polymers, drug delivery systems, and ROS regulators for MFNPs.

CONCLUSION

This paper set out to investigate how does the size of the NP impact the growth and stress responses of bacteria when exposed to an AMF. The results demonstrate that the 10 nm SPION was the most effective dimension of IONPs to utilize to cause bacterial apoptosis, as it had the highest IONP absorption rate, the highest ROS concentration, and maintained its temperature within hyperthermic range. These results supported the hypothesis, as the superparamagnetic properties did help the IONPs enter into the bacterium to induce thermal and oxidative stress. The results also demonstrate that the size of the NP contributed to the bacterial stress response and death observed in experimentation.

ACKNOWLEDGEMENTS

I would like to thank my mentor Mr. Johnston and Mr. Sheka for their guidance, materials, and feedback. I was also like to thank Professor Addicott of biological sciences for providing a lab space and materials at the department of biological sciences at the University of Calgary.

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Being in love with the wonders of the smaller world of Biology, Zainab Hakim is a student researcher who currently studies at Nelson Mandela High School. She does a majority of her research at the University of Calgary under the direction of her mentor, Chad Johnston. Most of her research focuses on the properties and applications of nanoparticles in biology and medicine. Her most recent research regarding the propoerties of magnetic nanoparticles in relation to bacterial stress responses has garnered recognition at the regional, provincial, and national level. From a young age, Zainab has always been intrigues about the whys of the body and therefore has led to a passion to pursue medicine in her academic endeavours. She does this through volunteering, STEM outreach, competitions, networking, and doing research. Although her academic passions are a large part of her life, she always takes time to enjoy outdoor activities with her friends, such as biking, hiking, and swimming. Aside from her future aspirations in medicine, one of Zainab's forseeable academic goals is to participate in international research collaborations.