Decreased *Staphylococcus aureus* activity in the presence of iron oxide magnetic nanoparticles

Nhiem Tran[1] and Thomas J. Webster[2]
[1] Department of Physics, Brown University, Providence, RI 02912. USA.
[2] Division of Engineering and Department of Orthopaedics, Brown University, Providence, RI 02912. USA.

Statement of Purpose: *Staphylococcus aureus (S. aureus)* is a common type of bacteria that has a growing ability to resist current antibiotic treatment and has been, thus, causing a tremendous health care problem [1]. As an example, 14% of current orthopedic implants now become infected. As an alternative strategy to antibiotic treatment, the goal of the present in vitro research was to determine the bactericidal effect of iron oxide nanoparticles on *S. aureus* activity. It is believed that magnetic nanoparticles can be controlled by an external magnetic field to inhibit *S. aureus* immediately at infectious sites. In this study, decreased *S. aureus* activity in the presence of citric acid coated iron oxide magnetic nanoparticles is reported for the first time.

Methods: Magnetite nanoparticles were prepared by a wet chemical method similar to a previously described method [2]. Iron (II) chloride and iron (III) chloride with a molar ratio of 1:2 were dissolved in deoxygenated water in the presence of HCl. The resulting solution was added drop-wise to a NH_4OH solution under vigorous stirring and nitrogen flow to obtain magnetite nanoparticles (Fe_3O_4). The solution was added with citric acid and heated for 15 minutes under vigorous stirring. Maghemite nanoparticles (Fe_2O_3) were obtained from magnetite by aeration in boiling water at low pH.

S. aureus were obtained in frozen form from ATCC (ATCC 25923). The bacteria were plated on an agar plate before incubation for 16 hours in a standard culture environment (humidified 37°C, 5% CO_2, 95% air). A single colony of *S. aureus* was selected using a 10 µl loop (Sigma) and inoculated into centrifuge tubes containing 5 ml of tryptic soy broth. Bacteria in centrifuge tubes were then incubated at 37°C under agitation at 200 rpm for another 16 hours. At that point, the bacteria solution was diluted in tryptic soy broth to an optical density of 0.52 at 562 nm using a microplate reader (SpectraMax300, Molecular Devices). According to the standard curve correlating bacteria number with optical density, this value was equivalent to 5×10^6 cells/ml. The cells were further diluted in tryptic soy broth to 5×10^4 cells/ml before being added to a new centrifuge tube at 3ml/tube.

Concentrated Fe_3O_4 and γ-Fe_2O_3 nanoparticles in solution were added to bacteria tubes at different doses (low ~ 8 µg/ml, med ~ 80 µg/ml, high ~ 800 µg/ml). A tube of bacteria without nanoparticles served as a control. The Fe_3O_4 and γ-Fe_2O_3 solution was also added to tubes containing only tryptic soy both at the same concentration as above and served as particle controls.

After 3 hours of incubation, 100 µl of the bacteria suspension were transferred into a 96-well plate. A live/dead assay was performed according to manufacturer's instructions (Live/Dead BacLight. Invitrogen L7007). Briefly, two solutions containing SYTO 9 dye and propidium idodide were mixed and diluted with double distilled water before being added to a bacteria solution at 100 µl/well. The plate was incubated at room temperature in the dark for 15 minutes. The fluorescence intensities of live and dead bacteria were measured using a microplate reader and were divided and reported as a ratio of live/dead bacteria. The experiment was repeated three times.

Results: TEM images demonstrated that magnetite and maghemite nanoparticles with diameters ~ 20nm were successfully synthesized. The nanoparticle solution was stable during the experimental period.

The TEM images of bacteria in the presence of Fe_3O_4 nanoparticles demonstrated the existence of nanoparticles inside the bacteria.

The results from the live/dead assay demonstrated that after 3 hours, the ratio of live/dead bacteria was significantly lower in the solution added with the highest dose of Fe_3O_4 and γ-Fe_2O_3 compared to the control samples as well as the low and medium doses samples (Figure 1).

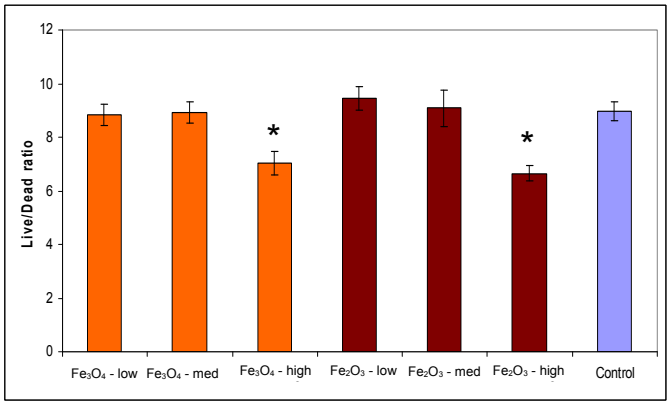

Figure 1. *S. aureus* live/dead ratios in the presence of Fe_3O_4 and γ-Fe_2O_3 nanoparticles. Low ~ 8 µg/ml, Med ~ 80 µg/ml, High ~ 800 µg/ml. Data = mean +/- SEM; N = 3. * $p < 0.05$ compared to the control (no particles).

Conclusions: Fe_3O_4 and γ-Fe_2O_3 nanoparticles were successfully synthesized and characterized in this study using TEM. The live/dead assay showed that at the highest dose of iron oxide, the growth of *S. aureus* was significantly inhibited compared to the control samples. The bactericidal effect of iron oxide nanoparticles could be due to the generation of reactive oxygen species. The fact that nanoparticles were found inside bacteria could also contribute to the killing of *S. aureus*. Further studies also should investigate the bactericidal effect of Fe_3O_4 and γ-Fe_2O_3 nanoparticles on other types of bacteria for widening these novel antibacterial properties of iron oxide particles.

Acknowledgements: The authors would like to thank the Herman Foundation and Vietnam Education Foundation for funding.

References:

[1] Grinholc. M, et al. *J. Photochem. and Photobio.* **90**(1) 2008, pp. 57-63.
[2] Y.S. Kang, S. Risbud, J.F. Rabolt, P. Stroeve. *Chem. Mater.* **8** 1996, pp. 2209-2211.