Decreasing Biofilm Formation Through the Use of Magnetic Nanoparticles

Erik N. Taylor and Thomas J. Webster
Division of Engineering and Department of Orthopaedics, Brown University, Providence, RI 02917 USA.

Statement of Purpose: Infection has been reported on an array of implantable devices including joint prostheses, central venous catheters, endotracheal tubes, mechanical heart valves, and others [1]. The difficulty antibiotics have in treating resistant infection demonstrates the need for a different novel approach. The goal of this study was to explore the use of superparamagnetic iron oxide nanoparticles (termed SPION here) as a multifunctional platform for the inhibition of bacteria biofilm formation enabled by size-dependent material properties, surface modification, and coatings. It is believed that SPION could be used for various infection related applications. In this study, SPION were coated with Dimercaptosuccinic acid (DMSA) providing a multifunctional linker which can be used to conjugate various useful chemistries. Here, nanoparticles were coated with silver and zinc to improve antibacterial activity. DMSA can also be used to functionalize SPION via sulphur-maleimide interactions with antibodies such as anti-protein A which would target SPION to a *staphylococcus aureus* in biofilms. Once at the target site, SPION could provide antibiotic therapy, therapeutic feedback (via MRI), and even delivery of other drugs (Figure 1).

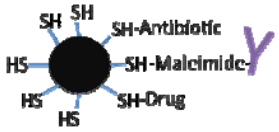

Figure 1. Schematic of multifunctional SPION. Free thiol groups of dimercaptosuccinic acid (DMSA) can be used to conjugate various useful chemistries such as antibiotics, antibodies, and drug molecules.

Methods:

DMSA-SPION Synthesis:
Particle synthesis was carried out by dissolving 1 mmol $Fe(acac)_3$ in triethylene glycol. SPION formation occurred during reflux at 273°C for 30 minutes. After cooling to room temperature, SPION were precipitated with ammonium sulfate in the presence of a magnetic field, and coated with dimercaptosuccinic acid (DMSA-SPION).

Antibacterial Conjugation to SPION:
Silver nitrate or zinc acetate was added to 1mg/ml of a stock SPION solution in ethanol at either 100 or 10 mg. Zinc and silver salt and nanoparticle mixtures were placed on a plate shaker at 100 RPM and precipitated in the presence of a magnetic field. This was repeated three times to ensure there was no non-magnetic metal salt remaining in the solution.

Nanoparticle Characterization:
This study determined the size and magnetic properties of the SPION used. Transmission electron microscopy (TEM) was used to determine particle size. Magnetic properties were verified qualitatively by placing a centrifuge tube containing SPION dissolved in ethanol on a plate shaker in the presence of a magnetic field.

Bacteria Assay
Bacteria utilized were frozen *Staphylococcus aureus* (ATCC #25923) stock cultured in Tryptic Soy Broth (TSB) at 200 rpm for 18-24 hours until late stationary phase was reached. Bacteria were diluted at a ratio of 1:100 in TSB or TSB containing coated SPION. The average bacteria density was analyzed by a spectrophotometer calibrated by a standard curve after 48 hours.

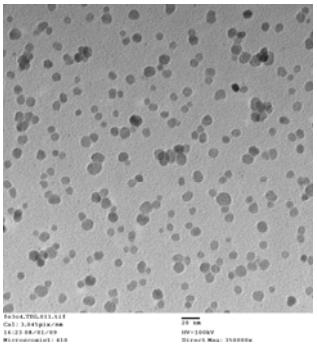

Figure 2. TEM analysis of SPION produced by refluxing $Fe(acac)_3$ in triethylene glycol. Average particle size is estimated to be 10nm.

Results:
Results of the present study demonstrated a simple method for the synthesis of water soluble SPION which could have multiple uses to decrease resistant infection (SPION shown in Figure 2). Moreover, bacteria results in the presence of antibacterial conjugated SPION for up to 48 hours indicated for the first time decreased cell density compared to no particles (0 mg/ml or control) (Figure 3).

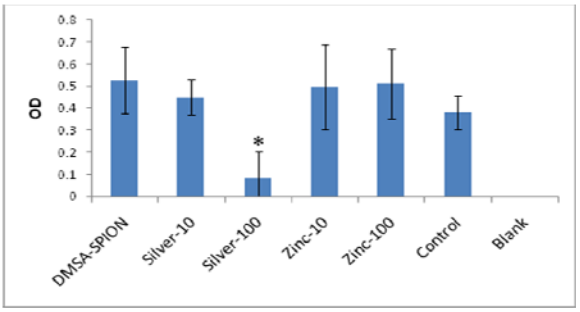

Figure 3. *Staphylococcus aureus* cultured in the presence of DMSA-SPION diluted 1:10 from 1 mg/ml stock solution. Bacteria were significantly inhibited by SPION coated with 100 mg silver (Silver-100) but not other nanoparticle solutions after 48 hours (10 or 100 indicates mg of salt added). *p values are significant at the 1% level compared to control values (N=3).

Conclusions: In this study, the possible minimal invasive treatment of device related infection was examined through the use of SPION. Results demonstrated that DMSA-SPION coated with silver were able to inhibit bacterial growth for 48 hours. Antibacterial properties of zinc were not observed possibly because the ion was not able to associate with the DMSA coating agent. In conclusion, these results help to confirm the hypothesis that SPION could have multiple uses during device related infections.

Acknowledgements: The authors would like to thank the Hermann Foundation for financial support.

References:
[1] Donlan RM. Emrg. Infect. Dis. 2001; 7,2.