Magnetic Nanoparticles and a Magnetic Field Designed for the Treatment of Prosthetic Infections

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Statement of Purpose: Bacterial infections of bone (called osteomyelitis) are of great concern to the medical community. In addition to bone, all medical devices are susceptible to microbial colonization after implantation. These infections are chronic because bacteria form a robust adhesion to surfaces, can become protected by a sticky slime matrix (called a biofilm) from the body's immune system (which would otherwise naturally clear the bacteria), and antibiotic treatments do not resolve such infections (due to antibiotic resistance). Here, the multifunctional properties of magnetic nanoparticles (termed here superparamagnetic iron oxide nanoparticles, or SPION) were explored for their antibacterial activity, magnetic properties, and drug delivery properties. This study provided a first step towards the development of a new type of pharmaceutical for orthopedic or other device related infection by demonstrating physical (magnetic) control of killing bacteria.

Methods:

Synthesis Method and Antibacterial Coatings of SPION

To produce SPION with coatings, synthesis was carried out using high temperature reflux of iron(III) acetylacetone in triethylene glycol, after which 4 mmol of dimercaptosuccinic acid (DMSA, Acros Organics) was added. Antibiotics gentamycin and vancomycin (Sigma Aldrich) were conjugated with Sulpho-SMCC (Pierce) or metals silver nitrate (Sigma Aldrich), zinc(II) chloride (Fischer), and iron(III) chloride (Riedel de Haen), were added directly to enhance antibacterial activity, and were purified with a magnetic field.

Methods Used for SPION Characterization

The physical properties of magnetic nanoparticles were determined using transmission electron microscopy (TEM), vibrating sample magnetometry (VSM), X-ray diffraction (XRD), and zeta potential measurements. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to quantify metal coatings, and Fourier transform infrared spectroscopy (FTIR) was used to analyze antibiotic coatings.

Analysis of Antibacterial SPION Properties

Staphylococcus aureus (*S. aureus,* #25923, ATCC) obtained as dry pellet was rehydrated in 6 ml Tryptic Soy Broth (TSB; MP Biomedical), incubated (37 °C, 5% CO_2, humidified environment), then mixed with equal proportions of 15% glycerol (Sigma), and frozen at −80 °C before inoculation.

Magnetic Biofilm Uptake

Prussian Blue (a histology stain creating a blue by-product in the presence of iron) was carried out to qualitatively determine the amount of iron deposited into biofilms. For this, biofilms were grown in vitro for 48 hours using overnight inoculums diluted at 1:100 in a 12 well plate on glass slides. SPION (100 µg/ml) were applied for constant 60 minutes with magnetic field exposure for 0, 20, 40, or 60 min.

Results:

Results of this study showed that the as synthesized SPION had a size of about 10 nm and saturation magnetization of 58.6 electromagnetic units per gram (emu/g). Results also showed that the conjugation of antibacterial agents to the SPION was successful (by FTIR and ICP-AES).

Interestingly, antibacterial properties were observed in SPION alone (Figure 1). Antibacterials also remained potent after coating. Moreover, it was determined that SPION penetrated the biofilm when applying a magnetic field (details shown in Figure 2 below).

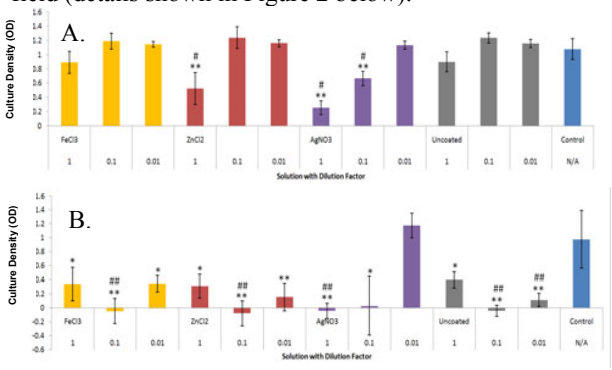

Figure 1. Inhibition of *S. aureus* with metal coated SPION without (A.) and after magnetic field exposure (B.). P values are significantly different at the 5% and 1% level respectively compared to control values (* and **), and when compared to 1 mg/ml uncoated nanoparticles (# and ##; N=3).

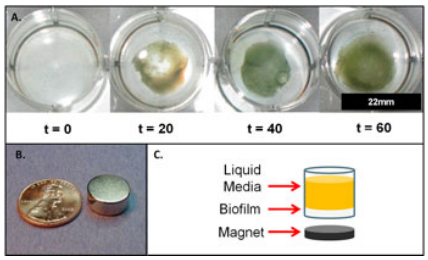

Figure 2. A. Magnetic uptake of SPION by S. *aureus* biofilms (48 hour) with Prussian Blue staining (to detect iron deposition). B. Permanent magnet used in this study (12.6mm dia. X 5mm thick magnet; 3761 Gauss; Neodymium). C. In this study, an in vitro *S. aureus* biofilm was first established in a polystryrene culture tray (12-well culture tray shown in A above). After addition of SPION, an individual magnet was placed below each well of the culture tray.

Conclusions: This study provided the first evidence that SPION are useful for treating various infections related to orthopedics. This research is promising, demonstrating the potential use of SPION for magnetic control, penetrating biofilms, and killing bacteria. SPION alone have interesting antibacterial and anti-biofilm properties which should be further explored.

Acknowledgements:
We acknowledge the Hermann Foundation for funding.