SYNTHESIS AND CHARACTERIZATION OF MAGNETIC NANOPARTICLES FOR DRUG DELIVERY TO CENTRAL NERVOUS SYSTEM
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INTRODUCTION
Approximately 50 million people in the United States are afflicted by neurological diseases, some of which are neurodegenerative. Neurodegenerative diseases involve the progressive neuronal death within the nervous system, due to overstimulation of neurons by excitatory neurotransmitters. An estimated $100 billion is spent in the United States on health care for Alzheimer’s disease (AD) alone. The country is therefore faced with an enormous economic burden due to AD and other related diseases [1-2]. There is no cure for AD and treatment is difficult to administer locally due to the blood brain barrier (BBB).

Magnetic nanoparticles (MNPs) are used as a vessel for CNS drug delivery, due to their ability to cross the BBB [3]. It is possible to target cell types via MNPs as microglia rapidly uptake them, allowing for localization of the drug delivery [4-5]. Silica-based magnetic nanoparticles are used to allow passive release of encapsulated drug, as well as controlled release via high-frequency magnetic field stimulation [6-7]. Accumulation and localization of magnetic nanoparticles in the brain can be controlled and localized by application of a magnet over the head and monitored by magnetic resonance imaging (MRI) [8-9].

OBJECTIVE
The goal of this project is to incorporate drug into MNPs and, in conjunction with MRI technology, release the drug in a localized manner. The project involves optimization of the MNP synthesis and release protocols to demonstrate uniform nanoparticle size, high drug load, and controllable release with effective doses.

SUCCESS CRITERIA
For optimal effectiveness of magnetic nanoparticles for drug delivery to the brain, the MNPs must be stable at room and body temperature to minimize unwanted passive drug release. The MNPs must also be less than 200nm in diameter to ensure their ability to cross the blood brain barrier. An increase of drug concentration in solution is expected after magnetic stimulation of MNPs.

METHODS
Initial studies are done with fluorescein. Relevant antioxidant drugs are substituted in later studies. Magnetic silica nanoparticle synthesis is done via the sol-gel method modified from the Hu paper [6]. The silica sol is prepared by mixing silicon tetraethoxide (TEOS), 2N HCl, and ethanol in a ratio of 2.25mL/500μL/600μL. This solution is aged for two hours and 40mL of a 0.25M solution of Fe(NO₃)₃•9H₂O is added, along with 2.5mg of the drug dissolved in 5mL of deionized water. The solution is brought to a pH of ~2.7 by the dropwise addition of 0.2M NaOH. MNP solution is aged for 24 hours and then dialyzed for 24 hours.

For scanning electron microscopy (SEM) imaging, small samples are dried at 80°C on gold film. Samples are tested for size distribution via light diffraction particle analysis. Further characterization is done via heat release studies and magnetic release studies. The heat release studies are done at 25°C (room temperature), 37°C (body temperature) and 80°C. Initial magnetic release studies are done at frequencies of 50kHz and 3kHz and amplitude of 10V. Further magnetic release studies are done via 7 Tesla MRI with gradient stimulation at 3kHz for 10 minutes. After all release studies, MNP solution is filtered via centrifuge to separate MNPs from supernatant. Supernatant is then analyzed using a spectrophotometer; fluorescence readings are taken for fluorescein-loaded particles, and absorbance readings for antioxidant-loaded particles. In vitro and in vivo studies are currently in initial planning stages.

RESULTS
MNPs are found to be stable at room and body temperatures (Figure 1). Analysis of particles via
SEM and light diffraction analysis determines that MNPs are formed at approximately 150nm, which is a suitable size for crossing the BBB (Figure 2). Magnetic release studies prove the possibility of releasing encapsulated drug via magnetic stimulation at various frequencies. Both applied frequencies (50kHz and 3kHz) result in approximately twofold increase in fluorescence intensity (Figure 3).

Figure 1. Fluorescein is released when exposed to temperatures > 80°C for at least 1hr as indicated by the increase in fluorescence of the MNP solutions (λ<sub>ex</sub>=485nm and λ<sub>em</sub>=535nm).

Figure 2. SEM images show average diameter of MNPS is ~150nm, which is suitable for crossing the blood brain barrier.

Figure 3. Fluorescein is released when exposed to magnetic fields at amplitude 10V and frequencies of 50kHz and 3kHz. Both frequencies, when applied for 1hr, result in approximately twofold increase in fluorescence intensity compared to the background control. Standard error bars are displayed.

**DISCUSSION**

Initial studies with fluorescein as the loaded sample drug prove MNPs can be synthesized effectively and the drug can then be released in a controlled manner via magnetic stimulation. Fluorescein is an effective sample drug due to its low cost and ease of imaging; well-defined excitation and emission wavelengths aid drug release quantification via spectrophotometry. MNPs loaded with several different antioxidants of interest – resveratrol and superoxide dismutase mimic – have been synthesized and are undergoing the same testing as the fluorescein loaded MNPs.

Heat release studies are proof of concept that drug is loaded into the MNPs during synthesis and can subsequently be released. Stability of MNPs reduces unwanted passive release and increases ability to control release. Success with magnetic release proves that MRI-triggered release of drug from magnetic nanoparticles is feasible. Preliminary drug release via magnetic stimulation is in therapeutically relevant doses, which is incredibly promising for clinical applications.

Several limitations were noted in this study. Optimal conditions for MNP synthesis and washing protocols took a long time to identify. Troubleshooting of drug release mechanisms also took a long time. It was determined that presence of nanoparticles in solution caused light scattering in the spectrophotometer, resulting in imprecise measurements. Therefore, filtering of the nanoparticles is now used before taking measurements.

Ongoing and future work includes testing with drug of interest – superoxide dismutase mimic, which has shown promise in treating neurodegenerative diseases. *In vitro* studies must be done to ensure minimal cytotoxicity from silica nanoparticles, although silica is known to be biocompatible. *In vivo* studies will confirm effectiveness of drug release, as well as aid in determining protocols for specific localization of drug release.

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**REFERENCES**