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Midpoint Progress Report to the FUS Foundation for a High Risk Research Grant

Project title: Enhancement of FUS Mediated Delivery of Stem Cells to the Brain

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Project Dates 2/1/15-1/31/2016

The goal of our project is to determine if magnetic attraction of super-paramagnetic iron oxide nanoparticle (SPION) labeled stem cells will enhance their delivery to brain after focused ultrasound (FUS) mediated opening of the blood-brain barrier (BBB).

Our first objective was to determine the parameters for our device (a small animal MRI guided FUS apparatus from Image Guided Therapy (IGT) that is installed on our existing 7T Bruker MRI, Figure 1) to transiently disrupt the BBB, allowing stem cells to enter the brain from the blood. We have successfully sonicated young adult rats with evidence of BBB opening radiologically with enhancement of the target region with gadolinium on post-sonication MRI. As expected from published studies we have determined a correlation between the intensity of sonication and the size and intensity of the brain region with gadolinium enhancement. Histological evaluation of these animals has also shown a correlation between regions of gadolinium enhancement on MRI in vivo, and staining with Evan's blue dye (a marker for disruption of the BBB) in post mortem brain tissue (Figure 2), allowing us to refine the sonication parameters to maximize BBB opening without signs of tissue damage such as hemorrhage.

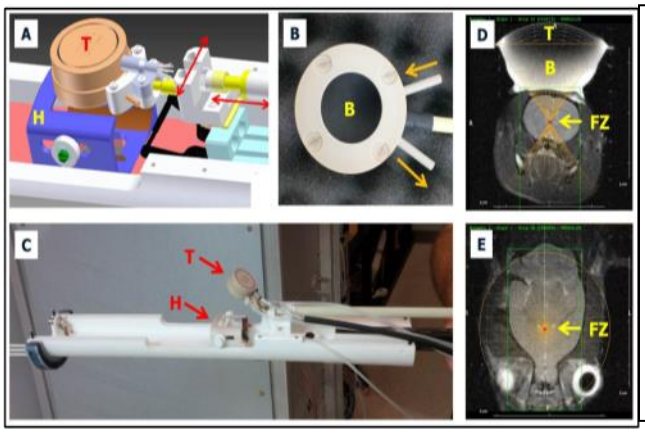

Figure 1. MR guided FUS system: (A) Schematic representation of the animal holder (H) and FUS transducer (T). (B) Underside view of the 8-element, annular array. (C) Animal holder 'H' and transducer 'T' assembly that is inserted into the bore of the MR scanner. (D – coronal view and E- axial view) Representative images from the MRgFUS user interface, indicating how FUS exposure is targeted in the brain of a rat. Locations of the transducer 'T', bolus 'B' and the focal zone 'FZ' of the FUS beam are indicated.

Prior to the initiation of our current study, the only previous published work where stem cells were delivered to the brain after FUS mediated BBB disruption (D), utilized injection of cells directly into the carotid artery of rats. In an effort to determine if FUS could accomplish this goal in a less invasive and safer manner, our study utilizes intravenous injection (IV) (rat tail vein). Our preliminary experiments have detected human neuroprogenitor cells (hNPCs) in the brains of rats after their IV injection. Although relatively few cells were detected, they occurred ***only*** within the areas of sonication. This supports the concept that FUS mediated BBBD call allow stem cells to enter brain even after minimally invasive IV injection. This process was improved

by pre-treating animals with the vasodilator sodium nitroprusside, which reduces the number of injected stem cells that are trapped within the lungs, before they can reach the brain's arterial circulation.

We have also combined this protocol with the application of a strong external magnet (2 inch neodymium cylinder) to the head directly after IV injection of stem cells. The goal of these experiments is to determine if the application of the magnet and its corresponding magnetic field could enhance the retention of the circulating SPION loaded stem cells in the sonicated region of brain. To directly determine the effect of magnetic attraction, we injected a mixture of cells loaded with SPION with an equal number of cells loaded with similar but non-magnetic nanoparticles that lack an iron oxide core. The different types of nanoparticles were labeled with different colored fluorescent dyes so that each type of particle and cell could be clearly distinguished. In animals treated with only FUS mediated BBBD, but without the external magnet, equal numbers of SPION loaded cells and non-magnetic nanoparticle loaded cells were found in the sonicated regions of brain as well as a sample of blood. In animals that also had the external magnet applied to the head, the majority of the stem cells within the sonicated region of brain were loaded with SPION, demonstrating an effect of magnetic attraction. This effect was most pronounced in brain regions such as the cerebral cortex which are located close to the skull and surface of the external magnet where 80-90% of stem cells contained SPIONs. In regions deeper within the brain such as the striatum, the target for stem cell therapy of Parkinson's disease, the effect of the magnet was less robust (60-70% SPION loaded cells)(Figure 3). As with sonicated rats without the magnet, human stem cells were not detected outside of the sonicated region. These results illustrate the potential of magnetic attraction to enhance delivery of stem cells from the blood stream after FUS mediated BBBD. The smaller effect seen in deeper brain regions also illustrate the inherent limitation in the use of a large but conventional magnet, where magnet field strength drops off steeply with distance from the magnet surface. This project is now moving forward to address this issue through the generosity of Dr. Mark Lythgoe, who has loaned us a Halbach magnet array that he designed for the specific goal of providing enhanced magnetic attraction at a distance from the magnet surface. Experiments with the Halbach array are underway to determine its capacity to improve retention of SPION loaded stem cells after FUS mediated BBBD even in deep brain sonicated regions.

In March of this year we submitted a proposal to VA Research to continue and expand this project entitled "Enhancing Stem Cell Delivery for Parkinson's Disease". Although this proposal was generally reviewed favorably, the project it was not funded at that time. We are re-submitting this proposal in September to include the preliminary data we have now developed through our FUS Foundation grant supporting its feasibility, and are optimistic about possible funding.

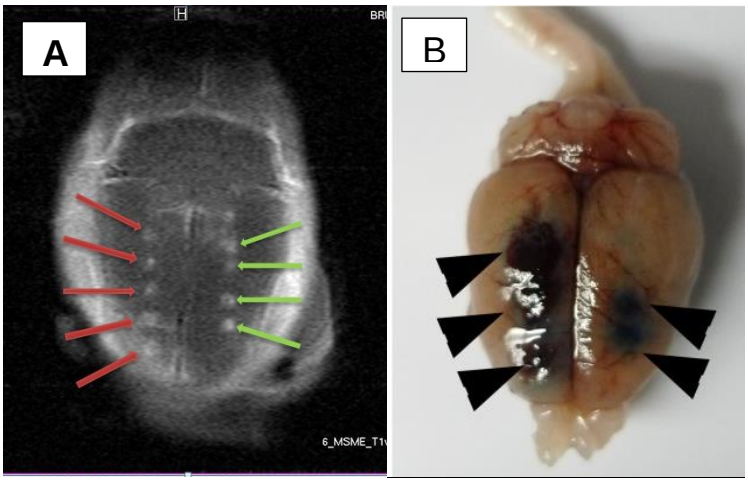

Figure 2. (A) MRI axial image (T2) of a rat brain after MRgFUS mediated BBBD showing gadolinium enhancement of sonicated regions red and green arrows .(B) Post mortem rat brain after BBBD and injection of Evan's blue dye which has extravasated into brain parenchyma giving a blue color seen in this view of the brain surface at left (arrowheads)

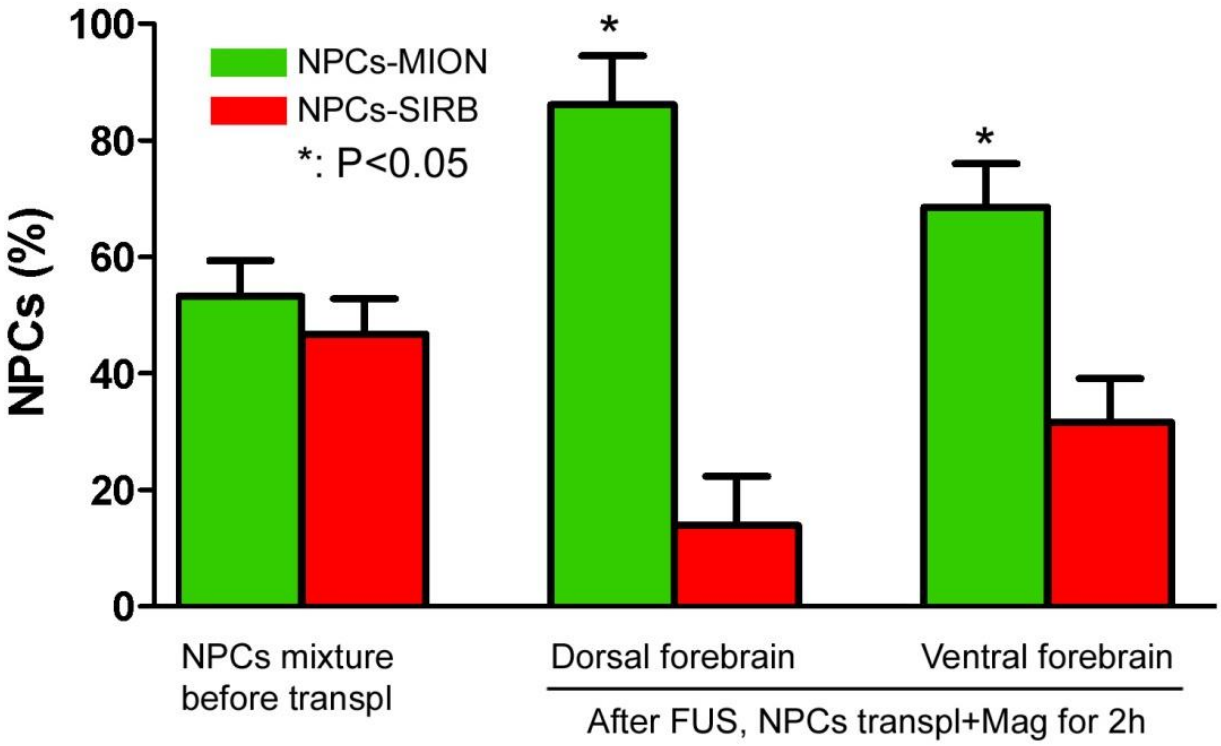

Figure 3. Quantification of nanoparticle loaded human NPCs detected in brain parenchyma in the region of sonication and MRgFUS mediated BBBD followed by the presence a large external magnet. The ratio of non-magnetic nanoparticle loaded cells (sans iron rhodamine B or SIRB) to SPION loaded cells (Molday iron oxide nanoparticle or MION) is not significantly different in a sample of blood. The significant majority of hNPCs in the region of brain sonication and BBBD followed by exposure to the magnet contain SPION. This ratio is greater in a dorsal region of sonication than in a ventral region at a greater distance from the external magnet.