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Final 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 was 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, to transiently disrupt the BBB, allowing stem cells to enter the brain from the blood. We successfully sonicated young adult rats with evidence of BBB opening radiologically with enhancement of the target region with gadolinium on post-sonication MRI. Histological evaluation of these animals has also shown staining with Evan's blue dye (a marker for disruption (D) of the BBB) in post mortem brain tissue without signs of tissue damage or hemorrhage (Figure 1A-C).

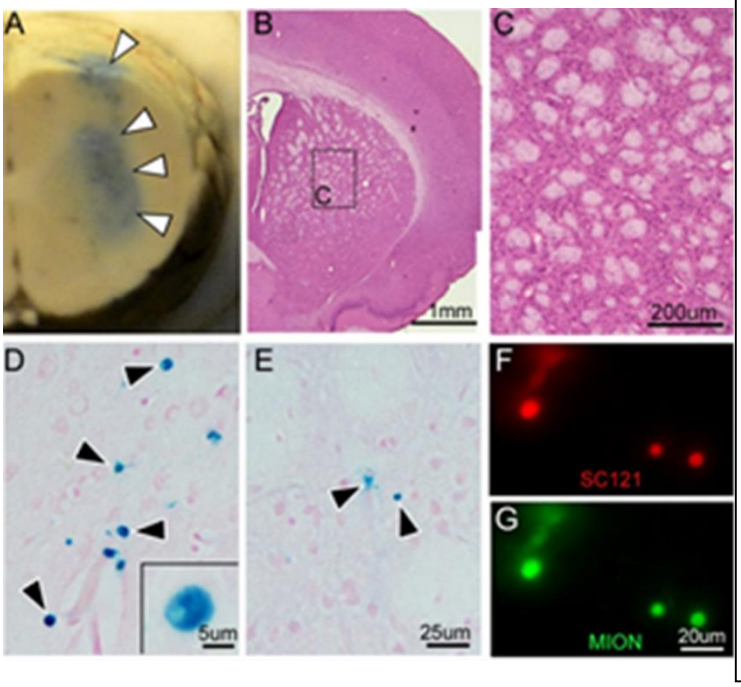

Figure 1: A) IV injected Evan's blue dye is visible on the cut surface of a fixed rat brain after FUS mediated BBBD both in a dorsal cortical region (single arrowhead) and in a deeper ventral striatal region (three arrowheads). B) H&E staining of these regions show normal cyto-architecture at low (B) and high (C) magnification. Iron containing cells are identified with Perl's stain (blue) in both cortex (D) and striatum (E) in regions of BBBD. These cells stain for the antigen SC-121 (F, red) confirming their human origin and contain fluorescent dye (green , MION,G) linked to the SPION

Prior to the initiation of our current study, the only previous published work where stem cells were delivered to the brain after FUS mediated BBBD, 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 utilized intravenous injection (IV) (rat tail vein). Our experiments detected human neuroprogenitor cells (hNPCs) loaded with SPION 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 allow stem cells to enter brain even after minimally invasive IV injection. The identity of the cells within brain as the originally injected cells was confirmed by staining with Perl's reagent for iron and by immuno-histochemistry with a human specific antigen (Figure 1D-G). 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 combined this protocol with the application of strong external magnets to the head directly after IV injection of stem cells. The goal of these experiments was 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 (MION, Evergreen) with an equal number of cells loaded with similar but non-magnetic nanoparticles that lack an iron oxide core, and were developed as a control (SIRB, red). 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. In initial experiments with a 2inch cylinder magnet 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 2A). As with sonicated rats without the magnet, human stem cells were not detected outside of the sonicated region. The smaller effect seen in deeper brain regions also illustrates the limitation of a conventional magnet, where magnet field strength drops off steeply with distance from the magnet surface, and was the rationale to assess two other types of magnets. Through the generosity of Dr. Mark Lythgoe we utilized a Halbach magnet array, designed to provide enhanced magnetic attraction at a distance from the magnet surface. We also assessed the effect of a larger (3 Inch) cylinder magnet which has significantly greater magnetic force. **Figure 2** illustrates a greater effect of both the Halbach array and the larger magnet on the retention of SPION (magnetic) loaded cells compared cells loaded with non-magnetic nanoparticles, particularly at the deeper regions. These cells are retained within the brain for at least a day after IV injection. With the ratio of magnetic cells compared to non-magnetic cells in the region of BBBD as an outcome measure, both types of magnets showed numerical superiority to our initial choice of the 2 inch cylinder.

Our proposal to VA Research to continue and expand this project using a rodent model of Parkinson's disease entitled "Enhancing Stem Cell Delivery for Parkinson's Disease" was reviewed and received a percentile rank of 3% and will be funded for two years based on the data developed through our FUS Foundation grant supporting its feasibility. A manuscript based on these results is currently in preparation.

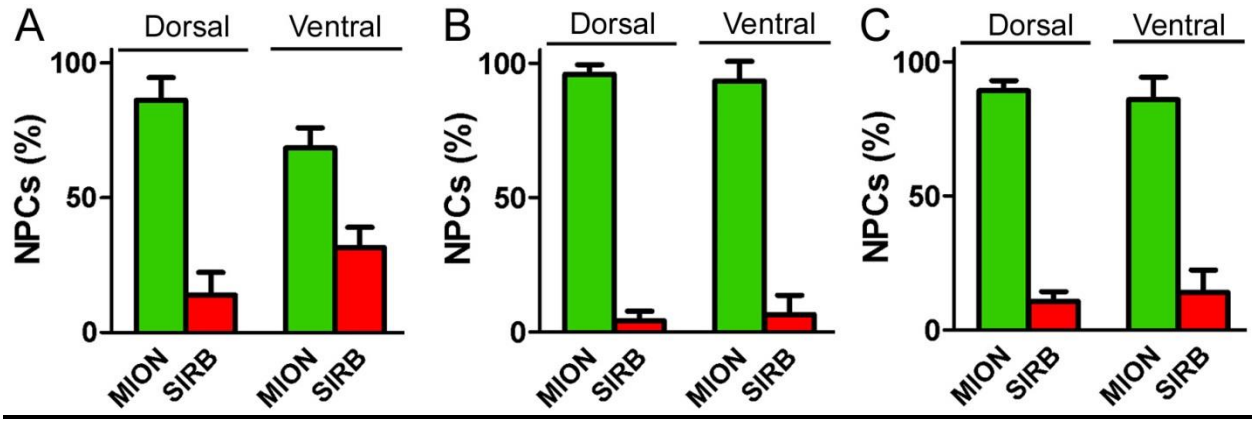

Figure 2. More intense magnetic fields further enhance magnetic attraction of SPION to deep brain regions after FUS mediated BBBD. Quantification of nanoparticle loaded hNPCs detected in brain parenchyma of rats in the region of sonication /MRgFUS mediated BBBD followed by the presence a **(A)** 2X2 inch cylinder magnet ; **(B)** a 3x3 inch cylinder magnetic and a Halbach magnet array **(C)** in place for 2 hours . The percentage of total cells that contain magnetic SPION loaded cells (Molday iron oxide nanoparticle or MION EverGreen, green) is numerically greater in all settings than that of non-magnetic nanoparticle loaded cells (sans iron rhodamine B or SIRB, red) with a trend toward a greater effect in deeper/ventral regions of BBBD with magnets with more intense field strength at a distance. Bars represent +/- SD from 3 animals with 223 cells counted in B, and from 2 rats where 345 cells were counted in C.