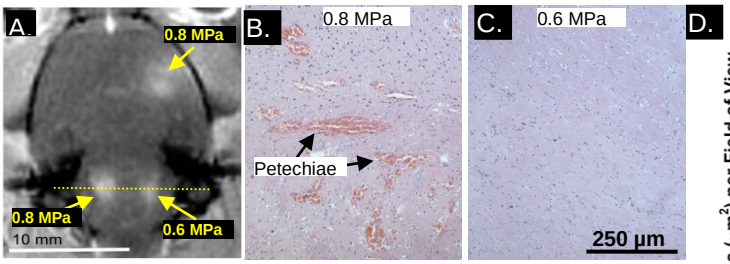
This FUS Foundation project consists of 2 specific aims:

Specific Aim 1: To determine FUS pressure and MB diameter combinations for reversible opening of the BBB in intracranial rat brain tumors. We will define FUS pressure thresholds for safe and reversible BBB opening to an MR contrast agent (gadolinium) in invasive intracranial brain tumors (human U251 glioblastoma xenografts) as a function of monodisperse MB diameter.

Specific Aim 2: To determine optimal conditions for delivering brain penetrating nanoparticle (BPN) formulations. The pressure thresholds from Aim #1 will be used as boundaries in determining optimal FUS and MB parameters for delivering BPN formulations across the BBB to U251 tumors.

For the first 6 months of this project, our work has been slowed considerably by technical difficulties with the small animal MR-guided focused ultrasound system. This system returned from the manufacturer approximately 5 months ago after having undergone a putative "upgrade", but it has not been working consistently since its return. At first, we encountered issues with the x-y-z stage translation. Although these issues were never completely resolved, we were able to perform some preliminary BBB opening experiments in rats. Unfortunately, a second problem with the system, related to the output pressure, was also revealed in these experiments. We are now in the process of working with the manufacturer to rectify this problem as well. Because of these techncial problems, progress has been slow and we anticipate having to request a no cost extension of the project. Nonetheless, we have performed a few experiments and are currently building a "table top" 1 MHz unguided focused ultrasound system that we can use for some high throughput experiments in the near future. The data we have are shown below.

Blood-brain barrier opening with MR-guided focused ultrasound and microbubbles. To determine how acoustic pressure affects BBB opening, we injected microbubbles, applied 0.01 sec bursts of 1 MHz FUS every 5 sec (0.2% duty cycle), and examined gadolinium diffusion by T1-weighted MR-imaging (Figure 1A). Contrast enhancement occurred above ~0.45 MPa. In histological studies, we observed petechiae at 0.8 MPa (Figure 1 B), but only rarely at or below 0.6 MPa (Figure 1C). Recently, we quantified petechial area as a function of distance from the center of the ultrasound focus. These data, which are presented in Figure 1D, illustrate that the threshold for petechiae creation is approximately 0.4 MPa. We have also shown that fluorescent PEG-



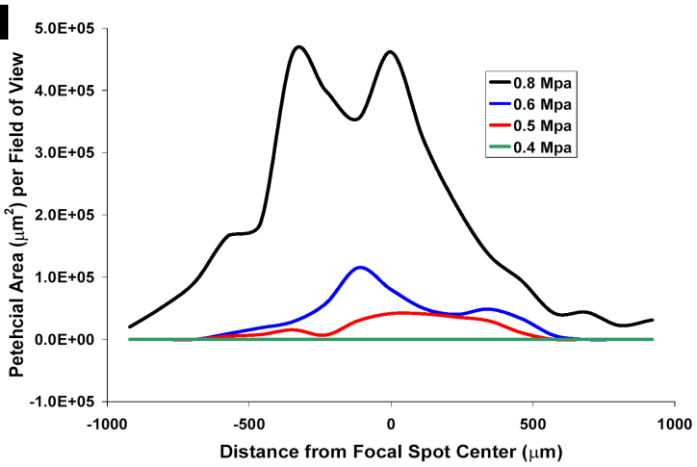


Figure 1. *A*: T1-weighted MRI showing BBB opening in focal spots after MB injection and 0.8 MPa (x2) and 0.6 MPa sonications. ***B and C***: Images from an H&E stained cross-section taken along the dotted line in panel A. Petechiae were evident at 0.8 MPa (**B**), but were rarely present or non-existent at 0.6 MPa (**C**). ***D***: Smoothed line graph of petechiae area as a function of position from the center of the FUS spot (i.e. x=0).

coated 50 nm tracer brain tissue-penetrating nanoparticles may be delivered across the BBB through 0.6 MPa and 0.4 MPa sonication sites (Figure 2). To our knowledge, this is the very first time that BPNs have been delivered across the BBB using MR-guidance. In recent studies, the delivery of these controlled release brain tissue-penetrating nanoparticles across the BBB was quantified using confocal microscopy. These results are shown in Figure 2D. These data appear to indicate that brain issue-penetrating nanoparticles can be delivered across the BBB using focused ultrasound at 0.4 MPa; however, it also appears that the extent of delivery is dependent on ultrasound pressure level, with greater delivery occurring at 0.6 MPa. Near term studies will now

entail determining whether reducing MB diameter variability will permit greater delivery at lower, and safer, acoustic pressure levels.

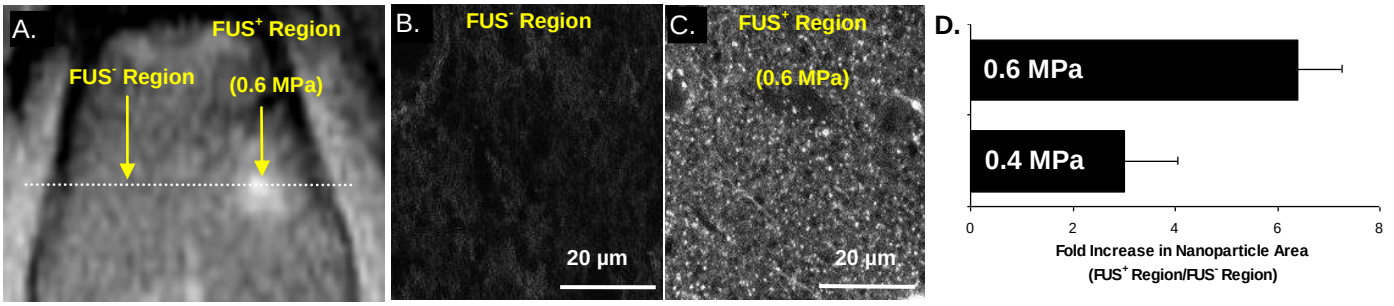


Figure 2. A: Contrast enhanced MR image showing BBB opening within a 0.6 MPa focal spot. Dotted white line denotes cross-sectional plane taken for images **B** and **C**. **B**: Confocal image of control region without FUS application (FUS^- Region). **C**: Confocal image of 0.6 MPa sonication region (FUS^+ Region) showing marked delivery of fluorescent 60 nm nanoparticles. Confocal imaging settings were identical for panels **B** and **C**. **D**: Bar graph of fluorescent nanoparticle delivery area in FUS^+ regions, given as a fold-increase over FUS^- regions. Metric was generated by thresholding identically-sized and –acquired greyscale images to black and white and then comparing white pixel fractions.