Novel liposome-microbubble complexes for administering therapeutic agents across the Blood Brain Barrier (BBB) in a targeted and minimally invasive manner

Amanda Halawani[1], Michael Valdez[2], Shelby Yuan[3], Theodore Trouard[2,4]

[1] NSF Research Experience for Undergraduates (REU), [2] Biomedical Engineering, [3] Keep Engaging Youth in Science (KEYS), [4]Department of Medical Imaging, University of Arizona, Tucson, AZ

Introduction

The ultimate goal of this project is to design a liposome-microbubble complex that can efficiently deliver drugs to the brain using focused ultrasound (FUS). Attaching therapy-loaded liposomes to microbubbles used in the opening of the blood-brain barrier (BBB) will greatly increase the efficiency of the technique for delivery of drugs to the brain while reducing systemic exposure. In previous studies, the BBB opening has been demonstrated with MRI and Gd-DTPA contrast agent (Gd) **(Figure 1).**

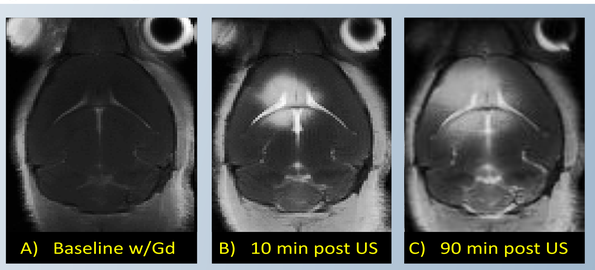


Figure 1. *T1-weighted MRI enhancement images. Signal increase from IP injection of Gd-DTPA (baseline). 10 and 90 minutes post FUS and 40 µL solution of microbubbles. Enhancement shows open BBB and diffusion of Gd-DTPA over time.*

Background

Mammals are equipped with a neurovascular unit called the BBB that isolates the brain tissue from the blood[1]. This barrier is comprised of blood vessels that contain endothelial cells with tight cell-cell junctions that allow very few substances to cross into the brain tissue (**Figure 2**). This includes the vast majority of drugs designed to treat neurological disease.

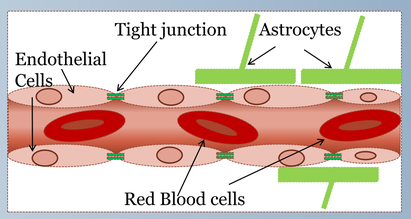


Figure 2*: Vessel schematic of the blood brain barrier (BBB).*

Methods

Methods currently used in drug delivery across the BBB are either invasive or have had little success. It has been previously demonstrated that the BBB can be temporarily opened by the cavitation of tiny lipid-coated, gas-filled microbubbles using FUS[2-4]. Microbubbles can be injected into the bloodstream and FUS is applied transcranially and focused to a region of interest in the brain. The temporary opening of the BBB allows therapeutics to pass into the brain tissue[5] (**Figure 3**).

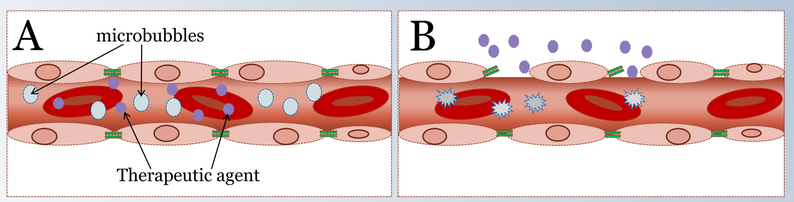


Figure 3*: Schematic demonstrating microbubble scatter and therapeutic agent within vessel before FUS (A). Temporary opening of BBB with therapeutics crossing the endothelial cell junctions after FUS (B).*

Methods

Liposome-microbubble complex - Liposomes were generated by extrusion of a solution containing 49% molar ratio of DPPC, 15% DSPE-PEG-maleimide, 36% cholesterol and 0.05 M Gd-DTPA in a phosphate buffered solution. For proof of principle experiments, Gd was used in place of a drug. 100 nm diameter liposomes were made using the extrusion process. Microbubbles were generated with a lipid monolayer solution synthesized of 8% DPPE-PEG 2000 and 92% DPPC lipids containing a conjugating agent, SPDP, to allow liposome conjugation about their surfaces **(Figure 4).** The microbubbles were 1 micron in diameter when shaken in per fluorocarbon gas at 4500RPM for 45 seconds. For MRI imaging, a cartridge was designed using SolidWorks to hold liquid samples centered within the MRI coil (**Figure 5**). This cartridge was printed on a Connex 350 3D printer (Objet Inc.) using MRI-compatible polymer.

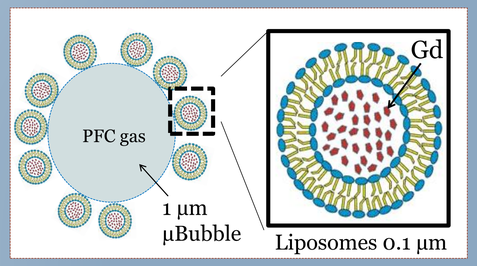


Figure 4*: Lipsome-microbubble complex encapsulating Gd. Image Adapted From: www.sciencedirect.com*

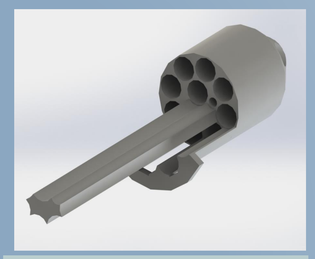


Figure 5: *SolidWorks rendering of cartridge able to hold 9 1mL liquid samples centered within the MRI coil.*

Results

Fluorescence imaging was used to demonstrate successful conjugation of liposomes about the surfaces of microbubbles coated with SPDP. A liposome sample was coated with fluorescent pink DiI which stains lipid membranes and introduced into two different solutions containing the microbubbles **(Figure 6).** In sample 1, coated liposomes were mixed with microbubbles containing the conjugating agent SPDP. In sample 2, coated liposomes were mixed with microbubbles not containing SPDP. Both samples received the same amount of coated liposomes and microbubbles.

Using a 7T MRI instrument, T1 measurements were made demonstrating successful encapsulation of a known concentration of Gd within liposomes (**Figure 7**). A sample containing Gd liposomes was heated. MRI images of the heated Gd liposomes have a higher T1 value confirming cargo release of known concentration of Gd (**Figure 8**).

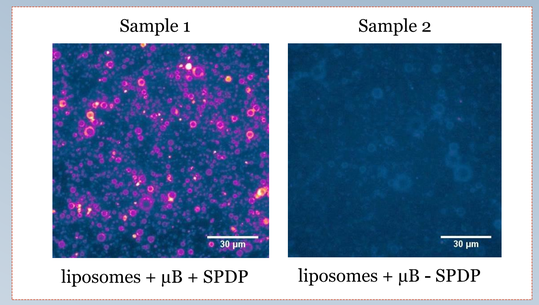
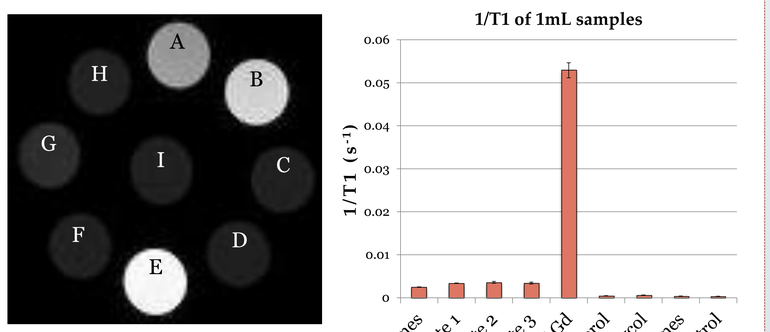


Figure 5*: Sample 1 shows a pink rings outlining the bubbles in solution. This confirms membrane-stained liposomes attached to the outer surfaces of SPDP-microbubbles. Sample 2 shows no pink outline, indicating the lack of stained liposomes on the surfaces of micobubbles without SPDP.*

Results



(A) Gd liposomes (F) Glycerol
(B) Dialysate 1 (G) Propylene Glycol
(C) Dialysate 2 (H) Blank Liposomes
(D) Dialysate 3 (I) PBS Control
(E) 0.01M Gd

Figure 7*: MRI image of cartridge containing samples. R1 values of each sample on the right.*

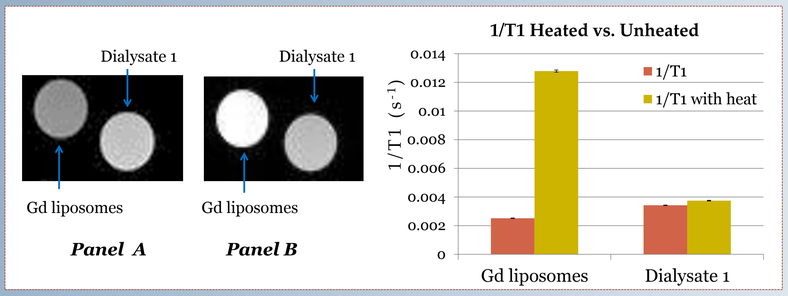


Figure 8*: MRI images of Gd-loaded liposome sample and Dialysate 1. Panel A shows Gd-loaded liposomes before heat is applied. Panel B shows Gd-loaded liposomes after 15 minutes at 69 °C. R1 values measured from the images are shown to the right.*

Conclusions

A liposome-microbubble complex can be achieved with encapsulation of a known concentration of a substance. Future work will use this complex with BBB opening for therapy of neurological pathologies such as Parkinson's and Alzheimer's. This complex will be especially useful for enhanced therapy throughout the body for tumors of vital organs with localized treatment.

Acknowledgements

Authors are grateful to the National Science Foundation for funding, the University of Arizona Physics Department, the laboratories of Dr. Romanaowski and Dr. Matsunaga, the BIO5 facility, and REU mentors; included Dr. Manne.

References

[1]Interlandi. Sci American. (2013)
[2]Hynynen et al. Acta Neurochir Suppl. 86:555-8. (2003)
[3]Mesiwala et al. Ultrasound Med Biol. 28(3):389-400. (2002)
[4]Camargo et al. Life Sci. 70(2):131-42. (2001)
[5] Geers et al. Journal of Controlled Release. (2011)