Characterizing the effect of agitation post - activation of lipid shelled microbubbles used for focused ultrasound

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Introduction

Blood - Brain Barrier (BBB)

- Selective and protective barrier from potentially harmful substances
- 98% of small molecules do not penetrate the BBB[1]
- Limits passage of therapeutic agents for neurodegenerative diseases including Alzheimer's Disease (AD)

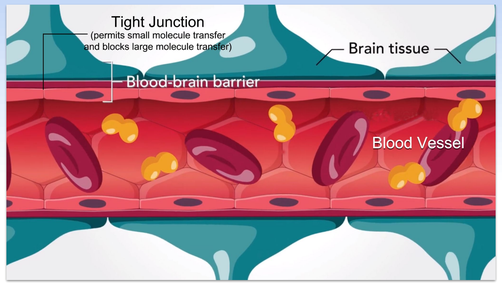

Figure 1. Blood - Brain Barrier.
The barrier needed to penetrate, MB infiltrates and expands to make the tight junction bigger and allow for more therapeutic drugs to enter the brain.

Focused Ultrasound Blood Brain Barrier Opening (FUS - BBBO)

- Used to enhance drug delivery, disrupt blood-brain barrier in a safe, reversible, and targeted manner[2]
- FUS promotes mechanical stresses termed acoustic cavitation within the cerebral vasculature upon introduction of lipid - shelled microbubbles through IV injection[3]

Parameters that influence Microbubble(MB) stability

- Parameters affecting the microbubble stability include composition of the lipid shell, gas core, and radial size[4]
- Agitation of the lipid shell that the MB is composed of is a method to create stable microbubbles through homogenization[5]

Objective

Understand the impact of agitation post-activation for the reuse of microbubbles.

Hypothesis

Microbubbles need to be agitated for stable formation through understanding concentration and MB diameter.

Materials and Methods

Activating Lipid - Shelled Microbubbles

1. Lipids were retrieved from the 4°C refrigerator.
2. Alternating sequence of vacuum and introduction of perfluorobutane was conducted (20 seconds each while retaining 40 seconds of gas at the end).
3. The Vialmix (R) was used to agitate and induce microbubble formation for 45 seconds.

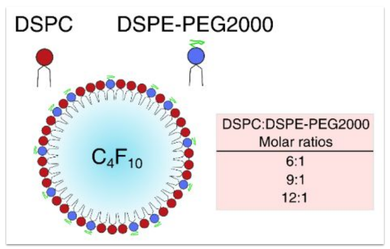

Figure 2. Composition of Microbubbles
The microbubble consists of an inner core composed of perfluorobutane and an outer shell composed of DSPC and DSPE - PEG2000[3].

Experiment

1. n=4 MB vials were activated and
2. n=2 were agitated for 45 seconds (agitation)
3. n =2 were left alone and only used when sizing (non - agitation)
1. All vials were sized on Day(s) 0, 3, 4, 5, and 7
2. Concentration and MB mean diameter was recorded.

Microbubble Sizing

1. 10 mL pipette was used to put isoton Ⅱ (diluent) into 4 cuvettes
2. Using one vial of isoton Ⅱ to clean unblock and flush
3. Inserting 2µL into one vial and then starting the program to size.
4. Select distribution of MB between 1 to 10 µm
5. Repeating this three times for each vial of microbubbles

Figure 3. Multisizer 4e Coulter Counter
Throughout the entire experiment, a sizing machine was utilized daily, and it was cleaned using Isoton Ⅱ. The sizing machine employs electrical zone sensing (EZS) technology for its measurements.

Figure 4. Vialmix ®.
The Vialmix pictured is used for standard activation of microbubbles.

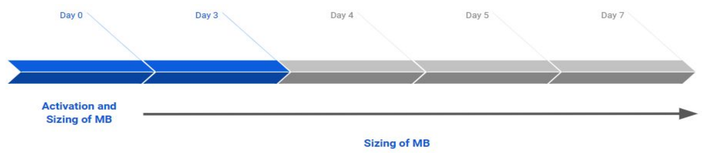

Figure 5. Experimental Timeline
Activating of MB happened 1 out of 5 days. Sizing of MB happened all days of the experiment over a week.

Results

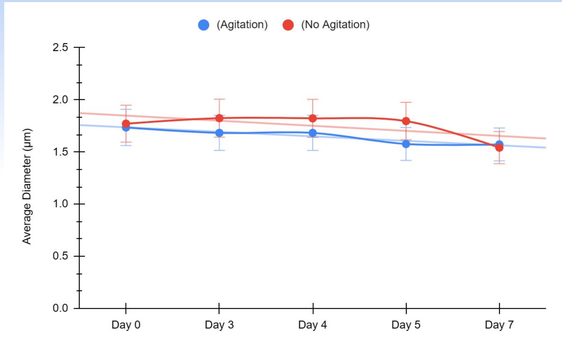

Figure 6. MB Diameter
Graph shows size of the MB telling us over time the average diameter decreases.

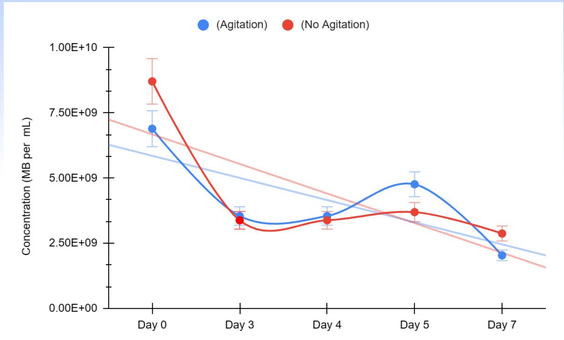

Figure 7. MB Concentration
Graph showing concentration of MB per mL shows a downward trend showing the concentration decreases over time.

- D 0 - D 7. Agitated MB diameter decreased by 0.16 µm. Non - agitated decreased by 0.30 µm.
- D 7 ended with 1.527µm for agitated bubbles and 1.542µm for non agitated bubbles.
- D 0 - D 7. Agitated MB concentration per mL decreased by 4.85×10^9. Non-agitated decreased by $5.84x10^9$.
- D7 ended with $2.04x10^9$ MB per mL for agitated bubbles and $2.87x10^9$ for non agitated bubbles.

Discussion and Conclusion

- Microbubbles need to be in a certain range in order to be effective. It needs to be small enough to enter the BBB and large enough to expand the BBB and allow drugs to enter when combined with FUS. **(Fig. 1)**
- Agitation of MB seems to be not as important as both produced result similar to each other in the end(**Fig. 6-7)**
- Agitation post-activation did not imply significant difference in either parameter, mean was consistent and trends were similar **(Fig. 6-7)**
- Average diameter and concentration in both cases of agitation and non agitation decreases throughout **(Fig. 6 -7)** .

Ongoing & Future Work

- Seeing or finding a greater difference between agitation and non agitated MB
- Perform in vitro experiment to absorb cavitation of MB under FUS

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