WHAT'S THE BIOBUZZ?
A journal for students by students

Brains and Bursting Bubbles!

Key Terms

Blood Brain Barrier (BBB):
A highly selective border of cells around the brain that controls what can go into it from the bloodstream.

Lipids:
Compounds that do not dissolve in water and are oily to the touch (Ex: natural oils, waxes)

Phospholipids:
A type of lipid with a "head" that can dissolve in water and a "tail" that cannot.

***In Vitro*:**
Performed in a test tube, culture dish, or elsewhere outside a living organism.

***In Vivo*:**
Performed in a living organism.

Ultrasound:
Sound waves that are emitted at frequencies beyond what humans can hear.

Focused Ultrasound (FUS):
Technology that uses ultrasound to target deep tissue within the body without using incisions or radiation.

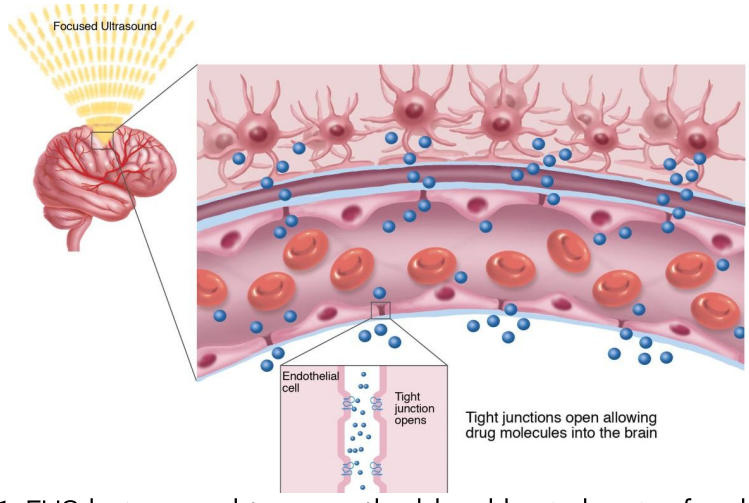


Figure 1: FUS being used to open the blood brain barrier for drugs.

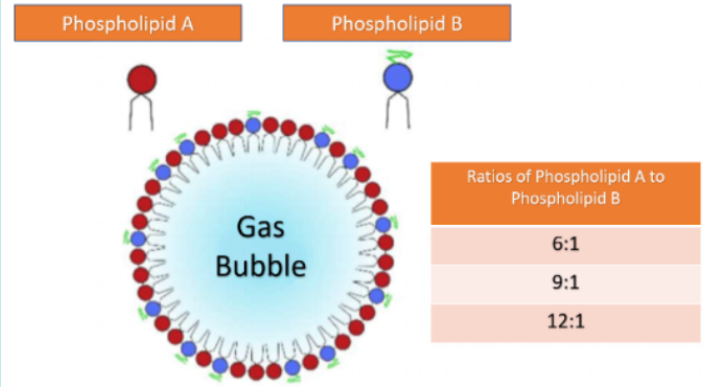
ABSTRACT

Researchers are studying ways to treat brain disorders by delivering therapeutic drugs into the brain, but there's one big problem: the brain is surrounded by the **Blood Brain Barrier (BBB)**. This barrier functions to protect the brain from being affected by toxins or germs that are in the blood. Yet, it also prevents other molecules, like certain drugs, from entering. Currently, researchers are trying to use **focused ultrasound (FUS)** and pre-formed microbubbles together to open the BBB. These microbubbles are tiny gas bubbles in a **lipid** shell that can carry drugs inside them. Their shells allow them to enter the BBB, and the ultrasound pulses push them through to the brain. Over time, their lipid shells expand and contract until they finally release the substances inside them.

The purpose of this experiment was to test the stability of a few kinds of microbubbles over longer periods of time during FUS exposure. They first activated the microbubbles and stored them for as long as three weeks. During the three weeks, they tested the microbubbles' stability as they changed the contents of the lipid barrier around each one. Researchers were able to choose the right combination of lipid to make the most stable shell for the microbubbles and found that the microbubbles were stable for up to 21 days after activation.

INTRODUCTION

The blood brain barrier (BBB) is important because it protects the brain from toxins and pathogens that can infect the brain. The BBB is semipermeable, which means that it allows only some molecules to pass through. Most drugs that are used to treat the brain can't pass through the BBB on their own. They must be covered in a **phospholipid** shell, called a microbubble, to pass through the blood brain barrier and reach the brain! But we need a way for the microbubbles to pass the BBB to their target location, open, and release the drug inside them!



Ratios of Phospholipid A to Phospholipid B
6:1
9:1
12:1

FIGURE 2 The microbubble is a gas bubble surrounded by a lipid shell. In this experiment, the shell was made of phospholipids A and B that were set up in 3 different ratios, as shown in the table.

Just like a magnifying glass focuses light into a single point, focused ultrasound (FUS) uses an acoustic lens to concentrate sound waves together at specific locations in the body. The **ultrasound** waves push the microbubbles through brain tissue until they reach the region where they can disperse the drug inside of them. There, they burst! This study researches the microbubbles stability over time, after the microbubble is created. The researchers looked at the microbubbles' stability microseconds after releasing the drug, and they investigated how storing the bubbles long-term would impact their stability. They hypothesized that the width of the BBB opening and microbubbles' ability to pass through the brain would not be affected by the storage time, as other scientists had assumed!

METHODS

Before testing the stability of the microbubbles, the researchers had to use computer simulations to pick the best ultrasound frequency to use for the FUS. Then, they moved onto ***in vitro*** testing to find the right ratio of lipids to use in the microbubble shell. They made a fake BBB, injected the microbubbles, and applied their chosen FUS pulse to see which type of microbubble shell was most stable (**Figure 2**). Finally, they ran experiments both in vitro and ***in vivo*** (in real mice) to see if those microbubbles were still stable in the BBB after being stored for 7, 14, and 21 days in the lab. They were interested in measuring the microbubbles' energy and hardness, along with the resulting BBB opening size.

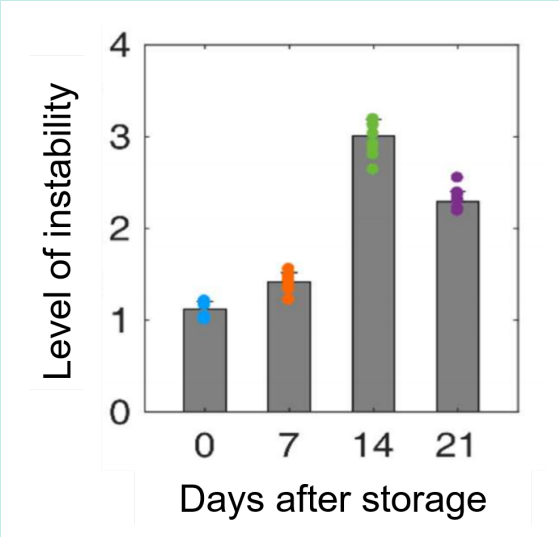


FIGURE 3 While the microbubbles all had relatively low levels of instability *in vitro* (which was a good thing), the level did rise for microbubbles stored for 14 and 21 days.

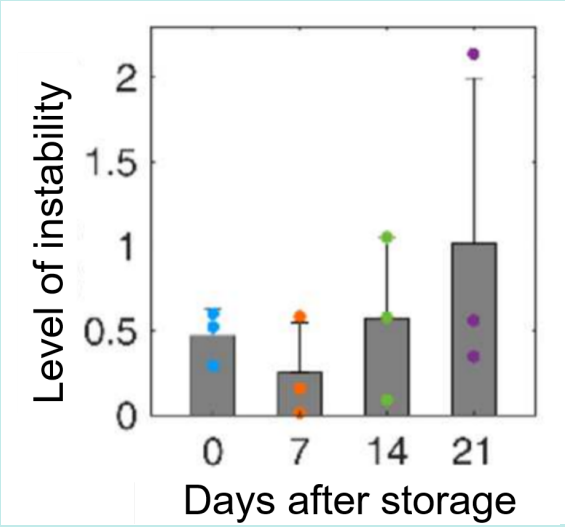


FIGURE 4 The *in vivo* results were similar to the *in vitro* ones as the microbubbles did mostly stay stable. Each dot within the bar graph represents the data for one mouse. Days 7 and 14 showed lower levels of instability than expected.

Error Bars- the lines sticking out of the bars on the graphs. The data on day 21 has large error bars. Large error bars mean the data points are far apart from each other and thus very uncertain. Day 21 had very high uncertainty, which made it seem less reliable!

RESULTS

In vitro, the 9:1 ratio of phospholipids created the most stable microbubbles. These microbubbles had the least energy, meaning they were least likely to suddenly burst. So, they were shown to be the best for targeted drug delivery. Even though the microbubble stability stayed mainly the same over the 21 days of storage, the microbubbles were most stable just after being created and on day 7 (**Figure 3**). This was because after day 7, some of the lipids in their shells gradually broke down while they were in storage. With the lipids breaking down, the lipid ratio changed, causing the shells to become too hard and break more easily.

For *in vivo* testing in mice, the same 9:1 lipid ratio was used for the microbubble shells. The results were similar to *in vitro*: microbubble stability stayed consistent over the three weeks of storage time! The microbubbles were more stable than expected after being stored for 7 and 14 days (**Figure 4**), but this was thought to be because higher amounts of microbubbles were injected into the BBB on those days of testing. Additionally, they found the size of the BBB opening was the same regardless of how long the microbubbles were stored.

DISCUSSION

- The researchers were able to confirm their hypothesis and show that microbubbles can be stored at room temperature and stably used up to 3 weeks after they are first produced.

- Stable microbubbles meant that the size of the BBB opening after the microbubbles were injected also stayed the same. A stable BBB opening size is necessary so that germs and toxins in the bloodstream won't seep into the brain as microbubbles are injected.

- The microbubbles used were only stable when their shells were made of very specific amounts of phospholipids. So, other scientists who want to use microbubbles with focused ultrasound for BBB drug injections must specifically create microbubbles for that purpose.

WHY DO WE CARE?

- This research supports a more cost-effective use of FUS and microbubbles. If researchers are able to produce many microbubbles at once and store them for later use, they won't have to waste time or money making new microbubbles every time they want to use them to deliver drugs. This will help immensely with treatments for disorders like Alzheimer's and Parkinson's disease, or even brain tumors!

- Some directions for future research might be to see how the amount of injected microbubbles affects their stability. Researchers also want to see how putting actual drugs inside the microbubbles will impact the results of this study, which only tested the stability of empty microbubble shells. Do you have any questions about these microbubbles that you want to explore?

Adapted by Hannah Glasser and Sammy Tavassoli from *Temporal Stability of Lipid-Shelled Microbubbles During Acoustically-Mediated Blood-Brain Barrier Opening* by Antonios N. Pouliopoulos *et al.*, Frontiers in Physics, 2020.