Development of Size-Isolated Microbubbles for Drug Delivery Applications

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Background

Microbubbles have wide spread applications in Biomedical Engineering, such as in ultrasonography, targeted drug therapy, molecule transportation, and more commonly for ultrasound imaging. Although microbubbles can be created in various diameters, the standard and most widely used diameter range is from 4-5 µm.

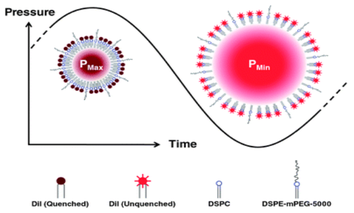

Figure 1: Representation of microbubbles oscillating with added pressure

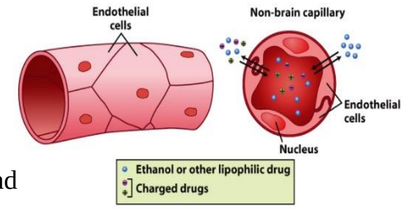

Figure 2: Composition of the blood brain barrier

Focused Ultrasound (FUS) is the use of an acoustic lens to concentrate multiple intersecting beams of ultrasound on a target deep in the body with extreme precision and accuracy. One such target are microbubbles, which oscillate in order to open the blood brain barrier to safely transport drugs and other molecules.

It is critical to effectively isolate microbubbles with specific diameters because different size microbubbles localize to different parts of the brain. In order to target the opening of the blood brain barrier to neural areas, microbubble sizes must be controlled.

Methods

For the purposes of this research, microbubbles of diameter 4-5 µm were isolated.
A solution of lipids was made, which included DSPC and DSPE-PEG2000

The solution was sonicated in water for about an hour at a temperature between 60 to 70 degrees Celsius. After being tip sonicated to finish dissolving the lipids completely, the solution was introduced to gas and the microbubbles were formed.

(a) (b) (c)

Figure 3: Tools used to form and isolate microbubbles
(a) analytic balance (b) sonicator (c) syringe

The microbubbles were then extracted by syringes and centrifuged 6 to 8 times to ensure only the microbubbles remained and no other solution was present.
All the microbubbles were transferred to one syringe, along with a solution of PBS and then centrifuged at 120 rpm to allow the larger microbubbles to sink to the bottom, isolating the 4-5 µm microbubbles. The number of times the microbubbles and PBS was centrifuged varied between 1-8 times.

Results

The isolated microbubbles were analyzed to see how precise the results were, and how effective the method was in creating microbubbles.
Microbubbles were successfully formed, but early trials produced samples with only about 25% of the microbubbles in the suitable 4-5 µm range. The quantity of suitable microbubbles formed were not nearly enough to use in ultrasound experiments for drug delivery in animals, because at least 80% of the sample must be within the target diameter range. As trials progressed, the quantity of microbubbles of 4-5 µm increased greatly.

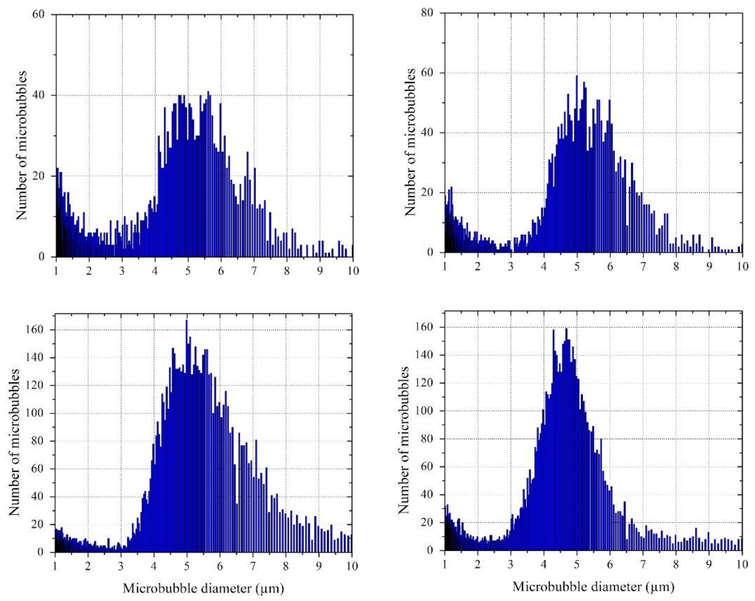

Figure 4: Results show the concentration of microbubbles formed at various diameter ranges. The target was to have the peak as narrow as possible and within the 4-5 µm range, and as tall as possible to create a large quantity of 4-5 µm microbubbles.

In the initial results, microbubbles diameters ranged from 2-8 µm. As the trials progressed, the range slowly began to narrow to 3-6 µm. Some trials produced very few microbubbles, and were not noticeable in a graph. Certain trials also failed to isolate the microbubbles, resulting in no distinction between the diameters.

Discussion

The purpose of the experiment was to form as many microbubbles as possible with a diameter as close to 4-5 µm as possible. After the formation of microbubbles and being extracted by the syringe, a large volume of foam was repeatedly picked up along with the microbubbles. This causes less microbubbles to be present at the experiment, and the data to be skewed. In order to produce less foam, the lipids must be completely dissolved in the sonicator before activating the microbubbles. In order to completely dissolve the lipids, they must be kept in the sonicator for longer, until there are little to no lipids left visible to the naked eye.

The results showed the diameters to still vary greatly. The diameter narrowed through each trial but the graphs, but aside from a few trials, most were too wide for successful results. Various trials contained no isolation of microbubbles and no peak was formed when graphing for the diameter range. However, the results did get more accurate over time due to less microbubbles being lost when recycling them through the syringes.

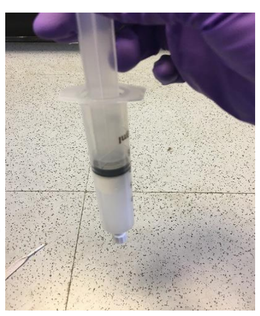

Figure 5: When extracting the microbubbles with the syringe, some microbubbles tend to stay left behind, while the syringe picks up foam, causing the results to be less accurate, and also fewer in quantity

Conclusion

Although some trials were able to produce microbubbles of 4-5 µm, majority of the results failed to show a successful isolation of 4-5 µm microbubbles. The data was not precise enough for the microbubbles to be used in Focused Ultrasound experiments.

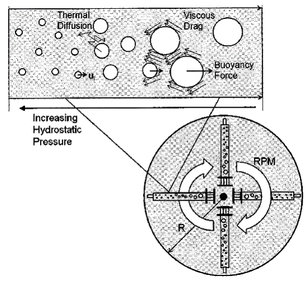

Figure 6: Image of syringes spinning in the centrifuge in order to isolate microbubbles by causing the larger microbubbles to sink to the bottom

The microbubbles produced would not have oscillated at the correct frequency to break the blood brain barrier because they were not in the correct diameter range. Since the blood-brain barrier is different sizes and lengths in various locations, the size of the microbubbles need to be within a specific range in order to open the blood-brain barrier at a targeted location.

To possibly increase effectiveness, the rpm of the centrifuge could be increased above 120. However, too much of an increase will cause the diameter range to decrease to 1-2 µm. Other diameter ranges could also be used to open the blood brain barrier, but the optimal range for microbubbles is at 4-5 µm. By testing different rpm and correcting the errors in creating microbubbles, it will soon lead to a more effective and efficient way in developing size-isolated microbubbles for drug delivery applications.

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