Microbubbles

AccuSizer®

Ultrasound is extremely sensitive to the presence of microbubbles so they are used as a contrast agent to enhance signal intensity in various ultrasonic molecular imaging procedures. This method is often called contrast enhanced ultrasound (CEUS). The particle size distribution and concentration of the microbubbles are critical to imaging quality and patient safety. The AccuSizer® is the preferred method to accurately determine the size and concentration of microbubbles used for molecular imaging.

INTRODUCTION

Many molecular imaging procedures with ultrasound rely on microbubbles to act as a contrast agent by increasing sensitivity between blood and surrounding tissue. Ideally the mircobubbles can be selectively adhered to regions of interest through surface modification. These targeted microbubble contrast agents (MCAs) are often fitted with a high-affinity targeting ligand. Once the MCAs are attached to the target they enhance the acoustic signal from pathological tissue.

Microbubbles for standard clinical imaging can be purchased, such as Definity®, and other researchers prefer to create their own microbubbles, typically for animal studies. Researchers in the Ferrara Lab, UC Davis Biomedical Engineering[1], make their own microbubbles for various studies.

One application of microbubbles is to study angiogenesis, the physical process of forming new blood vessels from preexisting vessels, a typical phenomenon in tumor development. Ultrasound imaging incorporating microbubbles can be used to detect and quantify angiogenesis by injecting the MCA intravenously. Measuring the rate of inflow contrast is related to tissue perfusion. The resulting images can be interpreted to study rate of flow, perfusion, and vascular architecture. The imaging could be further helped by targeting microbubbles to specific sites (e.g.; cancer in the body). Microbubble targeting usually is fulfilled by surface modification, often by adding peptides to the surface.

CRPPR is a Neuropilin-1 (NRP1) targeting peptide. NRP1 is a receptor for vascular endothelial growth factor and its expression is up-regulated in multiple tumor types, as well as on tumor vasculature. Microbubbles prepared at the Ferrara Lab were used

Figure 1. Lantheus Vialmix

for angiogene used for angiogenesis studies, in which the CRPPR microbubbles were used to evaluate tumor angiogenesis in a breast cancer model. The goals were to design a strategy for use of ultrasonic molecular imaging to assess local NRP1 concentration at the tumor. The bubbles were prepared using the process described below.

MICROBUBBLE PREPARATION

Lipids, which make up the microbubble membrane, were first dried under a vacuum, then resuspended with degassed microbubble buffer. Microbubble buffer was made by mixing 80% sodium chloride (0.9%), 10% propylene glycol and 10% glycerol, with the pH adjusted to 7.4 with sodium hydroxide. The dried lipid mixture was resuspended with microbubble buffer by first warming in a water bath, and then sonicating for 15 minutes until all of the lipids were resuspended in a clear solution of 2.5 mg per mL. The solution was cooled to room temperature, and 1 mL was pipetted into 2 mL glass serum bottles. The bottle headspace was purged using 10 mL perfluorobutane and stored at 4°C until needed for use.

One vial of the above solution was shaken on Vialmix® (Lantheus Medical Imaging, see Figure 1) to activate microbubbles.

The activated microbubble suspension was collected in a 3 mL syringe that was then filled with degassed Dulbecco's Phosphate-Buffered Saline (DPBS). The microbubbles were collected into a cake next to the syringe plunger, by using centrifugation at 300 RCF for 10 minutes. Next, the microbubble cake was resuspended with 2.5 mL DPBS, and bubbles larger than 10 µm were removed by two centrifugation steps: 1) 16 RCF for 1 minute, and 2) 45 RCF for 1 minute, and in both steps the bubble cakes are discarded while the liquid suspensions were collected. The resulting liquid suspensions went through a series of three centrifugation steps at 300 RCF for 3 minutes, to remove microbubbles <1 µm, in which the infranatant was removed, and the bubble cake was collected. The particle size and concentration were measured on the AccuSizer AD Figure 2 and were used within 3 hours of shaking. See reference 2 and 3 for more complete details of the microbubble preparation process.

Figure 2. AccuSizer AD

This process creates microbubbles similar to the FDA approved Definity perflutren lipid microsphere injectable suspension marketed by Lantheus Medical Imaging, but with a different lipid formulation.

The microbubbles were measured on the AccuSizer 780 AD system to determine the size and concentration. The particle size distribution of the control microbubbles is shown in Figure 3, and the particle size distribution of the CRPPR-microbubbles is shown in Figure 4.

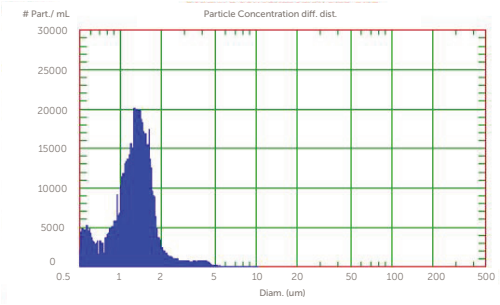

Figure 3. Non-targeted microbubbles size distribution

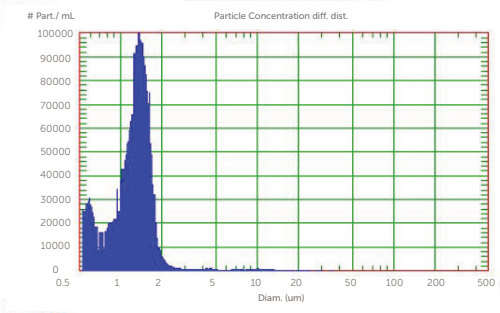

Figure 4. CRPPR microbubbles size distribution

CONCLUSIONS

For in vivo injection, the final size of the microbubbles is crucial. Adverse effects in research animals have been observed if the bubbles are larger than 10 µm in diameter, so having a tight size range between 1 – 2 µm in diameter is optimal for particle stability in circulation, as well as for animal safety. The AccuSizer has proven to be the preferred method of determining the size and concentration of microbubbles used as a contrast agent.

References

[1] The Dr. Katherine Ferrara Lab in the UC Davis Biomedical Engineering Department – Special thanks to Hua Zhang and Elizabeth Ingham for sharing this data and helping create this document

[2] Zhang., H. et.al., *Ultrasound molecular imaging of tumor angiogenesis with a neuropilin-1-targeted microbubble*, Biomaterials 56 (2015) 104-113

[3] Feshitan JA, Chen CC, Kwan JJ, Borden MA. *Microbubble size isolation by differential centrif-ugation.* J Colloid Interface Sci 2009; 329:316-24

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