Submission ID #: 62665
Scriptwriter Name: Madhulika Pathak
Supervisor Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=19112873

Title: Synthesis and Characterization of Multi-Modal Phase-Change Porphyrin Droplets

Authors and Affiliations:

Kimoon Yoo[1,2], Alexander Dhaliwal[1,2] , Juan Chen[1] , Paul S. Sheeran[3] , Gang Zheng[1,2]

1. Princess Margaret Cancer Centre, Toronto, Ontario, Canada
2. Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada
3. Philips Healthcare, Bothell, Washington, United States

Corresponding Authors:
Gang Zheng (gang.zheng@uhnresearch.ca)

Email Addresses for All Authors:
kimoon.yoo@mail.utoronto.ca
alex.dhaliwal@mail.utoronto.ca
juan.chen@uhnres.utoronto.ca
paul.sheeran@philips.com
gang.zheng@uhnresearch.ca

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interview Statements are read by JoVE's voiceover talent.

4. **Filming location:** Will the filming need to take place in multiple locations? **Yes,** 50 m indoors

Current Protocol Length

Number of Steps: 27
Number of Shots: 39

Introduction

1. Introductory Interview Statements

NOTE to VO talent: Please record the introduction and conclusion statements

REQUIRED:

1.1. This protocol is significant because it provides the complete procedure for creating droplets, from the lipid film, to lipid solution, to microbubbles, and then to droplets.

1.1.1. *2.1.2, 2.16.1, 2.19.1 and 2.24.2*

1.2. The main advantage of this technique is the simplicity for condensing microbubbles into droplets. Just by cooling and swirling, droplets can be made without the need for pressurization.

1.2.1. *2.22.1, 2.23.1*

OPTIONAL:

1.3. A visual demonstration is critical because a simple test to see if the microbubbles have been successfully condensed is by visually checking the change in translucency.

1.3.1. *2.24.2*

Introduction of Demonstrator on Camera

1.4. Demonstrating the procedure will be Kimoon Yoo, a Master's student in the Gang Zheng laboratory.

1.4.1. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Protocol

2. Droplet Formation

2.1. Using a decapper, remove the aluminum seal on the serum vial **[1]** and transfer 1 milliliter of the lipid solution to a 1.85-milliliter borosilicate glass sample vial with a phenolic screw cap by letting the lipid solution flow down the interior wall, without creating bubbles **[2-TXT].**

2.1.1. WIDE: Establish the shot of talent working on lab bench

2.1.2. Talent transferring lipid solution to glass vial **TEXT: 30 % (molarity) Pyrolipid**

2.2. With the 1.85-milliliter sample vial, prepare to flow in decafluorobutane gas into the sample vial headspace using the Gas Exchanger. Ensure that all the valves on the Gas Exchanger are properly closed **[1]** and the pump is turned off **[2]**.

2.2.1. Talent closing the valves on the Gas Exchanger

2.2.2. Talent turning the pump off

2.3. Open one Manifold Valve and carefully unsheathe the corresponding needle from the manifold **[1]**.

2.3.1. Talent opening the Manifold Valve and unsheathing the needle from manifold

2.4. Open Pressure Valve A and Pressure Valve B **[1]** and turn the Gas Cylinder Valve approximately one-sixteenth to one-eighth counterclockwise to partially open it **[2]**.

2.4.1. Talent opening the Pressure Valve A and Pressure Valve B

2.4.2. Talent turning the Gas Cylinder Valve counterclockwise

NOTE: Some shots of 2.5 and 2.6 are shuffled and the changes are updated as side notes by the authors

2.5. Open the T-Handle Valve **[1]** and uncap the sample vial with the lipid solution **[2]**.

2.5.1. Talent opening T-Handle Valve

2.5.2. Talent uncapping the sample vial with the lipid solution. NOTE: **This step was previously referred to as "2.6.1"**

2.6. Place the Manifold needle above the vial's liquid-air interface **[1]**, slowly turn the Air Regulator Valve clockwise until the Air Regulator gauge needle moves slightly from its resting position **[2]**, and let the perfluorocarbon gas gently flow into the vial headspace for 30 seconds, taking care to not create bubbles. Adjust the Air Regulator Valve if necessary **[3]**.

2.6.1. Talent placing Manifold needle above liquid-air interface inside uncapped sample vial, no gas flowing. NOTE: **This step was previously referred to as "2.6.2" or might be referred to as "2.6.2A"**

2.6.2. Talent slowly turning the Air Regulator Valve clockwise. NOTE: **This step was previously referred to as "2.5.2"**

2.6.3. NEW. Close-up of liquid-air interface inside the sample vial as the gas is flowing in. NOTE: **This shot might be referred to as "2.6.2B".

2.7. After 30 seconds, carefully and quickly cap the sample vial without moving the vial too much **[1]**.

2.7.1. Talent capping the sample vial

2.8. Close the Gas Cylinder Valve, the T-Handle Valve, Air Regulator Valve, Pressure Valve A, Pressure Valve B, and Manifold Valve **[1]**.

2.8.1. Talent closing the valves

2.9. Carefully sheath the needle **[1]**. Then, label the sample vial and seal the neck with wax film going clockwise **[2].**

2.9.1. Talent sheathing the needle

2.9.2. Talent labelling the sample vial and sealing the neck with wax film.

2.10. Store the sample vial in the dark and at 4 degrees Celsius for at least 10 minutes or up to 24 hours **[1]**.

2.10.1. Talent storing the sample vial in dark and in 4 degrees

2.11. Place about 100 grams of dry ice in an insulated container and place regular ice in another insulated container **[1]**.

2.11.1. Two containers, one with dry ice and other with regular ice

2.12. Retrieve a previously prepared decafluorobutane serum vial, two 1.5-inch 20-gauge needles, a 1-milliliter plastic syringe, a 200-milliliter container, metal tongs, and a thermometer **[1]**.

2.12.1. Decafluorobutane serum vial, two 1.5 inch 20-gauge needles, a 1 milliliter plastic syringe, a 200 milliliters container, metal tongs, and a thermometer

2.13. Place the sample vial with the lipid solution in the mechanical agitator and agitate for 45 seconds **[1]**. There should be a change in color and translucency **[2-TXT]**.

2.13.1. Talent placing the sample vial in the mechanical agitator and the vial getting agitated (shaken).

2.13.2. Added shot: Add a side-by-side picture for comparison: Pre-agitated sample vial (2.13.2A) on the LEFT and Post-agitated sample vial (2.13.2B) on the

RIGHT.
TEXT: LEFT: Pre-agitated, RIGHT: Post-agitated.

2.14. After the mechanical agitation, stand the sample vial right side-up, shielded from light, and start a 15-minute countdown to cool down the vial and size-select the microbubbles **[1]**.

2.14.1. Talent placing agitated sample vial in box for cool down and to block light

2.15. When the countdown has reached 10 minutes, fill a container with about 200 milliliters of isopropanol **[1]** and cool it to minus 20 degrees Celsius with dry ice using metal tongs **[2]**.

2.15.1. Talent filling the container with isopropanol

2.15.2. Talent cooling the isopropanol using metal tongs and dry ice

2.16. After the microbubbles have been size-selected for 15 minutes **[1]**, look for the size-selected partition inside the sample vial **[2-TXT]**.

2.16.1. Size-selected partition inside sample vial

2.16.2. Added shot: Add a side-by-side picture for comparison: Post-agitated sample vial (2.13.2B) on the LEFT and a 15 min size-selected sample vial (2.16.1) on the RIGHT.
TEXT: LEFT: Post-agitated, RIGHT: 15 min size-selected.

2.17. Keeping the sample vial right side-up, carefully uncap the sample vial **[1]**, and withdraw about 0.7 milliliter of the bottom partition with a 1.5-inch 20-gauge needle attached to a 1-milliliter plastic syringe. Ensure none of the top partition is withdrawn and do not flick the syringe to remove air pockets **[2]**.

2.17.1. Talent uncapping the sample vial

2.17.2. Talent withdrawing bottom partition with syringe

2.18. Aiming at the center circle on the rubber stopper, insert a different 20-gauge needle into a decafluorobutane serum vial while keeping the needle near the top of the serum vial to vent **[1]** and then insert the needle with the size-selected microbubbles **[2]**.

2.18.1. Talent inserting venting needle into decafluorobutane serum vial
Videographer: This step is important!

2.18.2. Talent inserting syringe with size selected microbubbles *Videographer: This step is important!*

2.19. Slowly transfer the size-selected microbubbles. Let the liquid gently slide down the interior wall of the decafluorobutane serum vial **[1]**.

2.19.1. Talent transferring the microbubbles by tilting the vial and letting the liquid to slide down *Videographer: This step is important!*

2.20. Once all the size-selected microbubble solution has been transferred, remove the needle with the syringe but keep the venting needle in to relieve negative pressure **[1]**.

2.20.1. Talent removing the microbubble syringe while keeping the venting needle in

2.21. Add small amounts of dry ice or room temperature isopropanol to the isopropanol bath to ensure the bath temperature is between minus 15 to minus 17 degrees Celsius **[1]**.

2.21.1. Talent adding dry ice or isopropanol to bath

2.22. With the 20-gauge venting needle inserted near the top of the serum vial, place the serum vial in the isopropanol bath, keeping the microbubble level below the level of the isopropanol but the vial neck above it, and intermittently swirl the serum vial for 2 minutes to condense the microbubbles **[1]**.

2.22.1. Talent inserting the serum vial with vent needle inside the isopropanol bath
Videographer: This step is important!

2.23. Do not swirl the serum vial continuously in the isopropanol and do not let the solution freeze. Swirl for about 5 seconds, and lift the serum vial out of the isopropanol. Check for ice nucleation, then resume swirling in isopropanol. If there is ice formation, swirl the serum vial in the air until it dissipates **[1]**.

2.23.1. Talent swirling the serum vial for few seconds, then lifting it and swirling again
Videographer: This step is important!

2.24. After the 2-minute condensation, remove the serum vial from the isopropanol bath and remove the venting needle **[1]**. The microbubbles should have been condensed into droplets, as indicated by the change in translucency **[2-TXT].**

2.24.1. Talent removing the vial from isopropanol bath and removing venting needle

2.24.2. Added shot:. Add a side-by-side picture for comparison: Pre-condensation vial (2.24.2A) on LEFT and Post-condensation vial (2.24.2B) on RIGHT.
TEXT: LEFT: Pre-condensation, RIGHT: Post-condensation

2.25. Wipe the serum vial, label it, and place it on regular ice in a dark, insulated container until ready for use **[1]**. Unopened droplets with an intact aluminum seal should be stable for up to 6 hours, as long as the melted ice gets replaced as needed **[2]**.

2.25.1. Talent wiping the vial, labelling it and placing it in an insulated container with ice

2.25.2. Unopened droplets with intact aluminum seal sitting in regular ice, in a Styrofoam container

2.26. When ready to use, remove the aluminum seal with the decapper **[1]**.

2.26.1. Talent opening Styrofoam container, retrieving droplet vial from ice, and removing aluminum seal with decapper

Results

3. Results: Characterization of Porphyrin Droplets

3.1. After using this protocol, three samples of each pre-condensed, size-selected microbubbles and post-condensed droplet samples were sized on a Coulter Counter with a 10-micrometer aperture. The sizing data for each of the Pyro-lipid content formulations is shown **[1]**.

3.1.1. LAB MEDIA: Figure 4

3.2. The statistics based on the sizing data are shown here. Using a ratio of pre- and post-condensed mean diameters, the results showed that as the Pyro-lipid content increased, the concentration decreased and the mean diameter increased **[1]**.

3.2.1. LAB MEDIA: Table 1

3.3. The representative absorbance measurements of the 30% Pyro-lipid droplet sample is shown **[1]**. This showed that the intact assemblies have different optical properties, as reflected by different peak **[2]** compared to the individual, unassembled lipid components **[3]**.

3.3.1. LAB MEDIA: Figure 5

3.3.2. LAB MEDIA: Figure 5 *Video editor: Please highlight the blue peak corresponding to the x-axis value of 700*

3.3.3. LAB MEDIA: Figure 5 *Video editor: Please highlight the orange dotted peak corresponding to the x-axis value of about 670*

3.4. Representative fluorescence measurements of the pre-condensed microbubble **[1]** post-condensed droplet sample with 30% Pyro-lipid are shown here **[2]**, demonstrating different fluorescence peaks for intact samples and the disrupted form **[3].**

3.4.1. LAB MEDIA: Figure 6A

3.4.2. LAB MEDIA: Figure 6B

3.4.3. LAB MEDIA: Figure 6

3.5. Representative ultrasound images of the 30% Pyro-lipid droplet sample imaged at different pressures are shown **[1]**. At low pressures, only background signal from air bubbles stuck from the agar synthesis was observed **[2]**.

3.5.1. LAB MEDIA: Figure 7

3.5.2. LAB MEDIA: Figure 7 *Video editor: Please highlight first row of the figure, 7A and 7B*

3.6. At a slightly higher power, a few microbubbles were generated, which is demonstrated by the appearance of bright speckles **[1]**. More microbubbles were generated as the pressure increased **[2]**.

3.6.1. LAB MEDIA: Figure 7 *Video editor: Please highlight second row of the figure, 7C and 7D*

3.6.2. LAB MEDIA: Figure 7 *Video editor: Please highlight third and fourth row of the figure, 7E, 7F, 7G and 7H*

Conclusion

4. Conclusion Interview Statements

NOTE to VO talent: Please record the introduction and conclusion statements

4.1. It is important to remember that the condensation temperature here is optimized for this specific droplet shell formulation. Different shell formulations may require a different temperature.

4.1.1. *2.23.1*