Nanoerythrosomes: Preparation and Interaction with Amphiphilic Polypeptides
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Background to my Project
1. Liposomes are used as drug carriers, but have limitations since they do not have long circulation times and are degraded by phospholipases and the immune system. On the other hand, red blood cells (erythrocytes) are nature’s carrier and are extremely effective at evading the immune system. 2. Our lab has developed a method to add drug containing patches to liposomes. This is done using a novel polymer known as a polypeptide (a synthetic version of a polypeptide) to stitch together layers. Now we seek to use the same technology to create patches on emptied out erythrocytes (erythrocytes). This is the topic of my graduate student mentor’s thesis.

My Experiments: Optimizing Ghosts

Different hypotonic solutions were tested in order to find the conditions that would yield empty ghost vesicles. 0.1X PBS appeared to show empty vesicles. Different hypotonic solutions were tested in order to find the conditions that would yield the best method to view these erythrosomes?

HMP was next introduced to liposomes in PBS solution, and then studied with the microerythrosomes.

How I Emptied Erythrocytes

Whole bovine blood was centrifuged to separate erythrocytes from serum.

Red blood cells (RBCs) were exposed to hypotonic PBS (phosphate buffered saline) solution.

Cells were washed with the hypotonic solution to allow most of the hemoglobin to leak out.

Ghost vesicles were resuspended in 1X PBS solution.

Resulting cells are termed microerythrosomes (MERs), sonicated or extruded vesicles are termed nanoerythrosomes (NERs).

• Optical microscopy was used to view red blood cells and microerythrosomes.
• Cryo-transmission electron microscopy was utilized to characterize liposomes with and without HMP.
• Confocal microscopy was used to see vesicles dyed with Nile Red and DII.
• DLS was used to measure the sizes of microerythrosomes, red blood cells, and vesicles treated with HMP.

What I have learned and accomplished

• I have learned valuable techniques including dynamic light scattering, optical and fluorescent microscopy, and cryogenic transmission electron microscopy.
• I have learned how to work with bovine red blood cells, remove the serum, and understand the role of hypotonic solutions in emptying cells.
• I have learned how to make drug-containing liposomes.

How the Polymer interacted with Ghosts

HMP was added to liposomes in this image above, and small fragments can be seen.

The above images show different hypotonic treatments: a) 0.25X PBS, b) 0.06X PBS, and c) 0.1X PBS. Too low an osmotic gradient, no intact vesicles remain. 0.1X hypotonic PBS seemed to be optimal.

An interesting finding was that these blayer sheets (D). With the 0.06X PBS treatment, no intact vesicles were there: cryo-TEM images show sheets on image D to the left. The optical microscopy images of the same sample only showed debris (part B).

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My Project Objectives

• Can I empty erythrocytes?
• Can I make erythrocytes to a specific size range?
• How do I characterize erythrosomes using optical or electronic microscopy and dynamic light scattering?
• Can I make the first steps in using polypeptides to attach patches onto erythrocytes?

NERs or ghost vesicles from the left were treated with HMP. The samples showed that vesicles become much smaller, and the red background below indicated smaller fragments present.

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Sources

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