Interaction of Engineered Nanoparticles with Artificial Cell Membranes

Rachel Benton

Chemical Engineering, North Carolina State University

NNIN REU Site: NanoTech User Facility, University of Washington, Seattle, WA
NNIN REU Principal Investigator: Jonathan D. Posner, Mechanical and Chemical Engineering, University of Washington
NNIN REU Mentor: Charles Corredor, Chemical Engineering, University of Washington
Contact: rabento2@ncsu.edu, jposner@uw.edu, corredor@uw.edu

Abstract:

Understanding the potential toxicity of engineered nanoparticles (ENPs) is vital due to their presence in over 1300 commercial products (e.g. toothpaste, sunscreen, and anti-bacterial coating on blankets). To further evaluate the potential consequences of ENPs, research must be performed to examine the interaction and mechanistic behavior between these particles and biological systems. This study seeks to understand these interactions by characterizing the effects of ENPs on artificial cell membranes. Understanding the interaction of engineered nanoparticles with lipid bilayers is an important step toward predicting subsequent biological effects and facilitates the design of safe nanoproducts. We believe that under some conditions ENPs can passively translocate across, and cause nanoscale defects in, lipid membranes. In this work, we quantify the disruption of liposomes induced by ENPs using a dye leakage assay. We measure dye leakage from lipid vesicles loaded with carboxy-fluorescein dye that are exposed to a diverse selection of ENPs. The amount of dye released correlated directly to the level of interaction between nanoparticles and the lipid vesicles.

Introduction:

Engineered nanoparticles (ENPs) are being utilized in various commercial ventures and research studies. Because of the rapid growth in the field of nanotechnology, there is a high potential that at some point in these particles' lifespan, they will come in to contact with the environment and humans [1, 2]. As of yet, the effects of these particles on health and the environment are not sufficiently well known. While ENPs hold a great deal of promise, before reaping the benefits of nanotechnology, more extensive research must be done in the field of nanotoxicity to understand their potential risks.

Membrane permeability is a vital component of toxicity studies as the membrane controls what enters and exits a cell. Lipid bilayers serve as viable artificial cell membranes in that natural cell membranes are comprised of over 80% lipids [3]. These same lipids, due to their hydrophilic head and hydrophobic tail, will self-assemble into spherical liposomes. This study seeks to understand the interaction of nanoparticles with artificial cell membranes by characterizing the effects of ENPs on liposome permeability using a dye leakage assay of liposomes exposed to ENPs.

Preparation:

Phospholipids were suspended in carboxyfluorescein (fluorescent dye), forming lipid bilayers, then passed through a 100 nm polycarbonate membrane in the process of extrusion. This caused a homogenous population of liposomes to form. As the liposomes formed, they encapsulated carboxyfluorescein inside them. The resulting solution was washed using unltracentrifugation by forcing it through a 3kDa membrane to remove the excess dye from outside the liposomes. This left just the liposomes (diameter approximately 100 nm) loaded with carboxyfluorescein.

This liposome, with the entrapped fluorescent dye, was then exposed to target ENPs. These experiments focused on two types of silver (Ag) ENPs, each with a 20 nm core diameter; one coated with polyvinylpyrrolidone (PVP), and the other with sodium citrate (NaCT). If the nanoparticles caused a disruption in the lipid bilayer, then the dye encapsulated inside the liposome would leak out. This amount of leakage was measured using fluorescent spectroscopy.

The carboxyfluorescein was excited at 480 nm and emitted at 520 nm. Because of this, the intensity measurements from the spectrometer were taken at 517.5 nm. The percent of leaked dye was found using the following formula:

$$Percent\ Leakage = \frac{I - I_{min}}{I_{max} - I_{min}} \times 100$$

where I is the intensity after liposomes are exposed to ENPs, I_{min} is the intensity of the liposomes without any ENP exposure, and I_{max} is the intensity after the liposomes are treated with Triton-X, which is known to cause total leakage from the nanoparticles.

The percent leakage was measured for three different conditions: liposomes without nanoparticle exposure, liposomes exposed to Ag NPs coated with PVP, and liposomes exposed to Ag NPs coated with NaCT.

Results:

Figure 1 and 2 show the percent leakage induced by Ag NPs as a function of time for 112 and 5600 PPB, respectively. The average percent leakage of fluorescent dye from the liposomes exposed to Ag NPs for both coatings was consistently higher than the liposomes without exposure for all tests. This showed a clear interaction between the ENPs and liposomes. In Figure 2, at approximately 1500 minutes, a greater percent leakage for liposomes exposed to sodium citrate coated Ag NPs was evident. The leakage also increased with increasing concentration. These results suggest that the surface functionality is a critical parameter in governing the disruption of bilayers induced by ENPs.

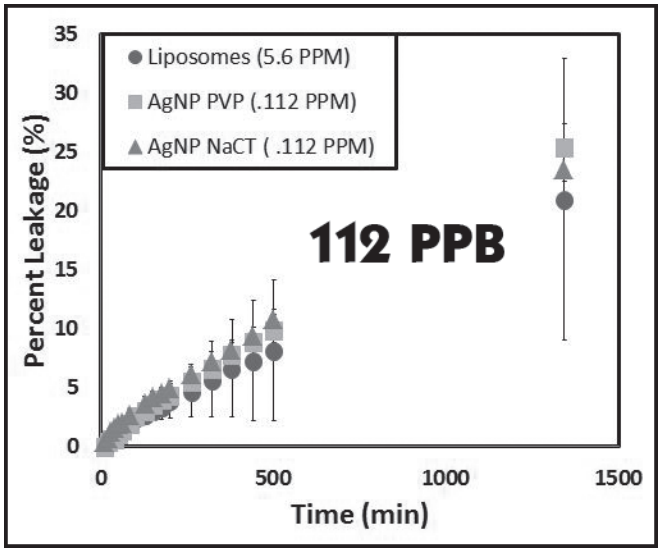

Figure 1: Kinetic measurements of liposomes after exposure to Ag NPs.

Summary:

These results show that ENPs disrupt bilayers and induce leakage of their internal contents. However, it cannot be inferred from this experiment whether or not these ENPs are toxic. Showing an interaction between ENPs and liposomes is an evidence that these ENPs are causing a disruption in the lipid bilayer and thus potentially in cell membranes, but the biomechanics of this interaction remain unknown. Understanding the interaction of ENPs with lipid bilayers is an important step toward predicting subsequent biological effects and facilitates the design of safe nanoproducts.

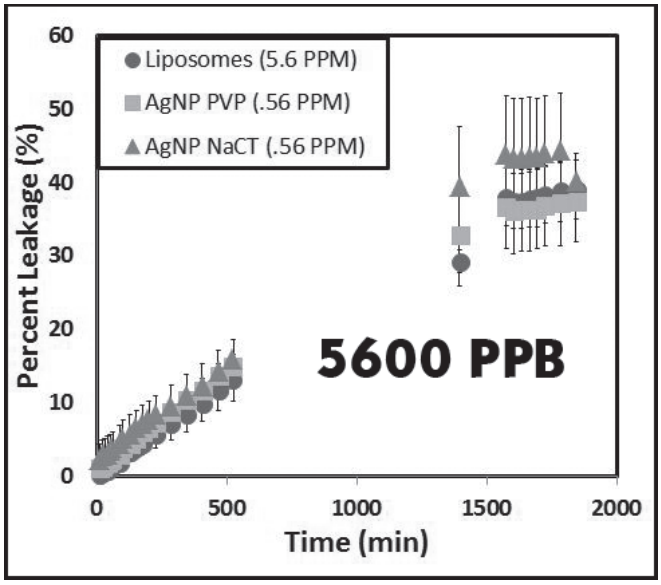

Figure 2: Kinetic measurements of liposomes after exposure to Ag NPs.

Acknowledgements:

I thank my Principal Investigator, Dr. Posner, and my mentor, Charles Corredor, as well as the rest of the Posner Research Group. I also acknowledge the National Science Foundation and the National Nanotechnology Infrastructure Network Research Experience for Undergraduates Program for the funding of my research. Thank you to University of Washington's Nanotech User Facility and to my program coordinator, Mack Carter. Finally, I would like to acknowledge Dr. Smith and Dr. Kavanagh from the Nanotoxicology Center at University of Washington with whom we collaborated with on this project.

References:

[1] Dean, L. Measurement helps solve nanoparticle toxicity challenges. Chemistry International, 34(4) (2012, July).

[2] Nel, A. E., Madler, L., and Velegol, D. Understanding the biophysicochemical interactions at the nano-bio interface. Nature. (2009).

[3] Roiter, Y., Ornatska, M., and Aravind, R. R. Interaction of nanoparticles with lipid membrane. Nano Letters, 8(3), 941-944. (2008).