

ABSTRACT

Cantley, Catherine S. M.S.A.B.E., Purdue University, August 2007. Enhancing the Sensitivity of Vesicle-Based Biosensors to Listeriolysin O. Major Professor: Jenna L. Rickus.

Of the foodborne illnesses reported, the most common sources of contamination leading to illness are *Campylobacter*, *Salmonella*, *Shigella*, and *Escherichia coli* 0157. Illnesses caused by *Listeria monocytogenes* are less common, but are responsible for the second highest mortality rate. Of the 2500 cases of listeriosis that occur in the United States each year, 20% result in fatality.

Listeriolysin O (LLO), a cholesterol-binding, pore-forming toxin, is the primary virulence factor of *Listeria monocytogenes* and a natural target for surveillance. Current detection methods for LLO are labor intensive and time consuming or rapid and insensitive to low concentrations. Mammalian cells are highly sensitive to the presence of pore forming toxins, such as LLO. However, their use in rapid detection biosensors is limited by stringent growth and storage requirements, short shelf life, and inherent fragility. To overcome hurdles of mammalian cell maintenance, we developed non-living nanocomposite materials that use liposomes, specifically large unilamellar vesicles (LUVs), to mimic biological function of cells.

Membrane characteristics, such as fluidity and charge density, significantly influence membrane permeabilization by pore-forming toxins. In this work, a factorial statistical design was used to systematically explore lipid composition of the liposomes. Although the addition of charged lipids, including phosphatidylinositol, phosphatidic acid, and stearylamine, were found to increase

LLO activity, a lack of repeatability in the liposome preparation methods precludes us from identifying which lipid composition provides the largest statistically significant increase in sensitivity. Liposome preparation procedures were investigated to ascertain the source of material variability. Multilamellar liposomes are well permeabilized by LLO and have very little variance associated with them.

A handheld device was developed for on-site testing. Ocean Optics fluorescence measurements instruments were selected based on a high degree of sensitivity, flexibility, and portability. Graphical user interfaces and programs were developed in both LabView PDA and Visual Basic.NET. USB communication channels have yet to be established.