ABSTRACT

Zhao, Jianxiu, Ph.D., Purdue University, August 2007. Sol-Gel Immobilized Liposomes as an Artificial-Cell-Based Biosensor for Listeriolysin O Detection. Major Professor: Jenna L. Rickus.

Listeriolysin O (LLO) is a pore-forming hemolysin secreted by the foodborne pathogen *Listeria monocytogenes* and is required for bacterial virulence. Current detection methods for *L. monocytogenes* are time-consuming, labor-intensive, and expensive. This is impractical considering the limitations of food storage. The general goal of this study is to develop a simple, inexpensive, and highly stable biosensor material that mimics existing whole-cell assays for LLO detection.

The first objective was to extend the stability of liposomes using an alcohol-free sol-gel route. The leaking stability of sol-gel encapsulated liposomes was reported by a fluorescence assay of carboxyfluorescein (CF). The effect of methanol on liposome stability was studied. This work demonstrated that the leakage of encapsulated dyes from sol-gel immobilized liposomes decreased largely with the removal of methanol formed during sol-gel process. The stability of liposomes was extended to at least five months with the alcohol-free sol-gel immobilization.

The second objective was to develop a biosensor for LLO detection using liposomes. The sol-gel immobilized liposomes served as cellular surrogates for membrane insertion and pore formation by LLO. Both free and immobilized liposomes responded to LLO at pH 6.0 with concentration dependent kinetics. The pore formation of LLO in liposome-doped silica composites displayed similar kinetic

curves as free liposomes but with slower rates. Immobilized liposomes could detect LLO in ~1.5 h using a steady state calibration and within 30 min using a kinetic calibration.

The third objective was to investigate the sensing system by developing a partial differential diffusion-reaction model. The pore formation and fluorescent dye release were described using a model based on reaction rate theory and evaluating LLO pore formation in free liposomes. Reaction rate constants were estimated by fitting the experimental kinetic data to the model. The reaction equations were then implemented in a finite element based model to investigate the LLO diffusion in liposome-doped sol-gel nanocomposites and the calcein release to the solution. With the developed model, diffusion of LLO and calcein was studied to determine the effects of important parameters on the sensor sensitivity.