Characterization of Biosensing Diacetylene Liposomes Fabricated Via Inkjet Technology

Cassandra J Wright, Michael A Evans, Danielle E Ebzery, Caroline E Hansen, Timothy W Hanks
Department of Chemistry, Furman University in Greenville, SC 29613

Statement of Purpose: Polymerized nanoscale vesicles such as polydiacetylene (PDA) liposomes have potential applications in a number of areas ranging from "smart" food packaging to bioterrorism and biomedical devices.[1] We have found that liposomes may be printed using a piezoelectric printer cartridge. This highly efficient technique produces nearly monodispersed vesicles and is superior to the common sonication method. PDA liposomes have been shown to detect a wide variety of stimuli including temperature, pH, mechanical stress and biological entities. These constructs are both exceptionally stable and undergo a vivid blue to red color change that can form the basis of a sensing system. The goal of this research is to design bacterial sensors with a fluorescence reporting system that is superior to the colorimetric response. This sensing platform could be used for detection of bacteria on wound sites, in food processing facilities as well as other applications.

Methods: "Ink" solutions were composed of 5mg of 10,12 pentatcosadynoic acid (PCDA), 10μL of an amino acid derivatized lipid solution, and 50-100μL of a fluorophore solution in 3.33 mL of 2-propanol. These were dispersed via a modified inkjet printer into DI water under stir followed by rotoevaporation and storage at 4 °C for at least 8 hours to allow self-assembly. They were then polymerized (254nm light; t=2min) to form PDA biosensors. Optimization parameters included testing "ink" solvents, printing rates, drop size, and temperature variation of the DI water (25°C, 40 °C, 60 °C).

Liposomes partially derivatized with amino acid (Arg, Trp, or Tyr; AA-PDA) and encapsulating a fluorophore (pyrenedecanoic acid, BODIPY558, BODIPY FL C11) were prepared similarly.

Lipolyzed polysaccharide (LPS) from three species of bacteria (E. coli serotype 026:B6, Salmonella enterica serotype enteritidis, and Pseudomonas aeruginosa serotype 10) were used. Each LPS was dissolved in a phosphate buffer (3mg/ml; pH = 7.4). 0.5mL PDA liposome solutions were exposed to 50μL LPS solutions for 24 hrs. (T=25 °C). Results were analyzed with an UV visible spectrometer (UV-Vis) as well as emission spectrometer (λex=348nm) and compared to a sample heated to 90 °C for normalization purposes.

Results: The inkjet printing method produced near quantitative yields of the biosensors (no aggregates detected filtering through a 0.45 μm filter). Fluorophores were successfully entrapped in the hydrophobic bilayers of liposomes having various amino acid surface decorations. The average size of AA-PDA was significantly larger (p=0.03) with similar polydispersity (p=0.08) compared to PDA liposomes as determined by DLS (Φ=142±1nm; PdI= 0.10±0.02 and Φ =128±20nm; PDI=0.10±0.02, respectively). Further, polydispersity decreased significantly compared to liposomes prepared using traditional methods (p=0.02). Optimized liposomes stability was found using 2-propanol solvent with a 3:2 PCDA:solvent ratio (15% red shift at day 14).

Figure 1 illustrates the advantage of using mixtures of fluorescent AA-PDA sensors over traditional PDA colorimetric detection. Instead of three separate experiments (with the three different AA-PDAs) showing a degree of color change, we are able simultaneously detect all three, giving a pathogen "fingerprint". These emission spectra differed to a degree significant enough to assign a distinct "fingerprint "to each type of bacterial LPS. In all cases, the arginine/1-pyrenedeconoic acid liposome displayed the most dramatic conversion to the red emissive state. The Salmonella LPS was emissive enough to possibly interfere with the height of peak 1 which could cause it to be larger than expected.

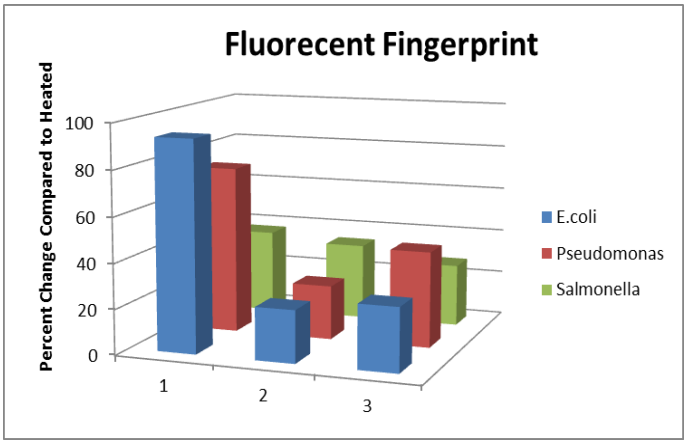

Figure 1: Fluorescent fingerprints from AA-PDA exposed to LPS solutions

Conclusions: Three different liposomes, each with a unique fluorophore and surface modification, resulted in a single solution detector system that provided discrimination between bacteria LPS tested. Ongoing work includes optimizing liposome surface decoration and improved selection of fluorophores (specifically those in the near-UV range) with the goal of extending the platform to four simultaneous outputs. Additionally, we are working to improve sensitivity and decrease the response time through liposome structural enhancements.

References: [1]Lasic DD. Handbook of Biological Physics 1995; 491-519.

Acknowledgements: This work was partially supported by the National Institute of General Medical Sciences (SC-INBRE grant number P20GM103499).