Title: Polydiacetylene liposomes as sanitation sensors for food processing

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Abstract

The objective of our research is to develop a method for rapid detection of microorganisms in food. Polydiacetylenes (PDAs) are conjugated polymers assembled from small molecules containing the two conjugated diacetylene moieties (DA). DA's undergo photopolymerization via 1,4-addition reaction to form alternating double-single-triple bonds along the backbone upon UV irradiation at 254nm. The highly conjugated material takes on a deep blue appearance. External stimuli such as heat, pH, mechanical stress and chemical solvents (Okada et al., 1998; Jelinek & Kolusheva, 2001; de Oliveira et al., 2012) lead to rapid color change from the initial intense blue color to red. It is believed that stress-induced twisting of the highly planar backbone lead to the color change of PDAs. These properties allow the PDA to act as a visual reporter component for a sensing system. PDA-based sensors which can detect ions, toxins, chemicals, or s have been reported. In an early example, Charych et al. (1993) developed a biosensor for influenza virus detection. Since then, great progress has been made in

developing PDA vesicle-based sensors. Sensors have also been developed that can rapidly detect cations (Kolusheva et al., 2000), proteins (Jung et al., 2010), melamine (Lee et al., 2011), bacteria toxins (Ma & Cheng, 2005), lipopolysaccharides (Rangin & Basu, 2004; Wu et al., 2011) and bacteria (Silbert et al., 2006; Nagy et al., 2008; de Oliveira et al., 2013). In general, those reported PDA sensors can be categorized into liquid platforms (suspended nanoparticles), crystal platforms and self-assembled mono/multilayers.

Our system uses a liquid-based platform prepared using a modified inkjet printing method with the ultimate goal of creating sensors for food pathogens. PDA vesicles were prepared and then characterized by dynamic light scattering (DLS) and UV/Vis spectroscopy. Eight common food sanitizers were tested with the PDAs to identify potential sources of false positives in processing plant applications. The seven commonly used sanitizers and one surfactant tested in this study were as follows: sodium hypochlorate (the Clorox company), BTF Iodophor sanitizer (National Chemical inc.), AFCO 4312 Vigilquat (Alex C. Fergusson), FreshFx AL (Synergy Technologies), FreshFx LP (Synergy Technoligies), TritonX-100 (Sigma-Aldrich) and Electrolyzed Water (Acid pH=2.96, Neutral pH=7.09 which generated from Hoshizaki water electrolyzer). Among the eight commonly used sanitizers and surfactants in food industry, PDA vesicles only responded with color change to Vigilquat and TritonX-100, which indicated a selectivity of PDA vesicles. Possible reasons of this phenomenon maybe the active component in Clorox™, electrolyzed acid water and electrolyzed neutral water which all include ClO^-. The ClO^- may repel the negatively charged PDA vesicles and therefore not interact with PDA vesicles. Iodine and hydriodic acid in Iodophor are neither positively charged nor hydrophobic and therefore cannot trigger a color transition. Organic acid such as citric acid, phosphoric acid, sulfuric acid and lactic acid constitute FreshFx LP and FreshFx AL. They are all hydrophilic and can lower the pH of solution but cannot twist the configuration of PDA vesicles and therefore no color change was observed.

We have also created a solid-based PDA platform on cotton, paper and also on agar that have given a color change from blue to pink after culturing with *E. coli*, indicating the potential application of liposome as indicators in the food processing environment.

This research is a collaboration with Dr. Hanks from Furman University, Dr. Pennington from Department of Chemistry, and Dr. Tzeng from Microbiology. Dr. Pennington's team has been working on absorbing PDAs on different matrices for detection such as cotton. Dr. Hanks' team has recently incorporated gold nanoparticles into PDAs to optimize their optical, magnetic and chemical functionality. We will continue to work on improving the sensitivity of the platform and applying PDAs in real food production environments.

Keywords: Polydiacetylene vesicles, PDA, Sanitizer, Food industry, Sensor.

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