Nanoengineering biomimetic nanobrushes for pathogen sensing

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PIs: **Eric S. McLamore[1]**, **Carmen Gomes[2]**
[1]Agricultural and Biological Engineering, Institute of Food and Agricultural Sciences, University of Florida
[2]Biological & Agricultural Engineering, Texas A&M University

PROJECT OVERVIEW

Food spoilage and foodborne infections are two major problems that affect the global food industry. The rich nutrient matrix and high water activity of fresh and minimally processed foods create a favorable environment for the proliferation of specific spoilage organisms (SSOs) as well as foodborne pathogens (FPs). According to the United Nations, the economic implications of these problems are stunning: approximately one third of the world's food production is lost annually; in a large extend due to food deterioration associated with microbial activity (FAO, 2011). In the US, foodborne diseases cause 1.2 million illnesses, 7,300 hospitalizations, 140 deaths, a cost of $1.6 billion, and a loss of 1,400 Quality Adjusted Life Years (QALYs) each year (Batz et al., 2012; CDC, 2011, 2012). *Salmonella* Typhimurium, *Listeria monocytogenes* and the "big six" diarrheagenic *Escherichia coli* pathotypes (STEC) are the most predominant FPs that persistently cause infections (Koluman and Dikici 2013).

The global impacts of this problem are even more alarming, as diarrheal and intestinal infectious diseases caused 38 million global cases of disease with 1.5 million deaths, leading to a loss of 84 million Disability Adjusted Life Years in 2015. The challenge of maintaining a safe supply of high quality food product while minimizing losses is exacerbated by the growing consumer demand for more natural, less processed products containing fewer preservatives (Yue et al., 2009; Banterle et al., 2013; Lê et al., 2013). This has global impacts, as production and export of high value commodities by developing nations must meet the stern quality and safety import requirements (labeling, certification and traceability) established in developed nations, in turn restricting the global trade of fresh produce (Unnevehr 2000; Quested et al., 2010; Engler et al., 2012). To meet the need of supplying fresh, high quality, and safe food to a growing world population, rapid and sensitive monitoring techniques are needed which can determine the presence of SSOs and FPs. Food safety biosensors are diagnostic tools that can be used to detect SSOs, FPs, and/or biomarkers of microbial contamination during harvesting, processing, storage, and distribution processes.

In the 60 days before the submission of this project overview, there have been 60 recalls issued by the FDA, and 32% of these are related to *Listeria monocytogenes*, which is significantly higher than the annual average of 17% reported by the USDA Reportable Food Registry (RPR, 2016). The food products range from ice cream, to juice, smoked salmon, fresh produce, and frozen vegetables. At this point it is unclear as to whether *L. monocytogenes* contamination in the US is on the rise, but these recalls highlight the importance of rapid tools that could be used to monitor pathogens such as *Listeria*. *To reduce the economic and quality of life burden from listeriosis, there is a pressing need to develop low-cost, rapid biosensors that can discriminate L. monocytogenes in a wide range of food environments.*

Project objectives

The goal of this collaborative research is to develop rapid, low cost *L. monocytogenes* biosensors based on composites of stimuli-responsive polymer nanobrushes and aptamers.

Objective 1: Synthesize and characterize stimuli-responsive polymer brush-aptamer conjugates for improving capture of *L. monocytogenes*.

Objective 2: Test and validate hybrid metallic nanoparticle sensors with nanobrush capture structures.

Objective 3: Validate nanobrush sensors on paper and plastic for application in food safety monitoring.

Methods

In the first year, we conducted fundamental research on the arrangement and architecture of nanobrush-aptamer assemblies by testing various chemical linkers and operating conditions. We also tested the combinatorial effect of hybrid nanometal-nanobrush materials on sensor performance. These first studies allowed us to determine the optimum arrangement for nanobrush actuation and pathogen capture, while maintaining electrical properties of the sensor film. We have applied this fundamental knowledge to design, build, and test biosensors on low cost platforms such as paper and plastic. For creating plastic electrodes, we have integrated state-of-the-art systems to fabricate laser-inscribed graphene electrodes on flexible, environmentally degradable materials such as polylactic acid or cellulose paper. We have recently been applying these sensors by testing performance and validating electric signals with standard analytical techniques. We are challenging the sensors in relevant food environments (e.g., temperatures ranging from -1.5 to 50°C and pH ranging from 4.3 to 9.6). We have recently added a probabilistic modeling component that will help analyze the uncertainty when applied for food safety using the standard regulations set by the Food Safety Modernization Act (FSMA) together with national early warning/monitoring systems such as PulseNet.

In addition to the research activities above, we have disseminated the findings through three outreach/community engagement activities. Namely: 1) Annual Biosensors Summer Camp for pre-collegiate students. The objective of the camp attendees is to build/test a working biosensor using common household items. The modules were designed, facilitated and assessed by PhD students. The goal of these experiential learning modules is to make technology more approachable for STEM students and non-experts in the community. This effort serves to recruit future STEM students and to increase public knowledge of research into technology related to agriculture; 2) K-2 Plants Club: We developed a program for local Kindergarten youth together that consisted of hands-on activities, lessons, and educational materials related to agriculture and food safety; 3) Biosensor workshop for 6^{th} grade girls. Three workshops were offered as a part of the Expanding Your Horizons camp and were well received by the attendees. Graduate students and faculty directed and moderated the workshop. The attendees filled out a short survey about the workshop that was highly ranked with comments.

Field of impact

The combinatorial effect of stimulus-response polymer nanobrushes functionalized with DNA aptamers for selective pathogen targeting is expected to significantly improve the field of pathogen detection. The project will produce an optimized method for capture and detection of bacteria based on aptamer functionalized stimulus responsive polymer nanobrushes, i.e., biomimetic nanobrushes. The material will be used to fabricate a rapid, sensitive tool, which can easily be integrated into food processing monitoring. This sensor can be used to limit waste of food by developing new monitoring plans for food contact surfaces and other priority areas within a processing plant.

Collaborations

We have initiated a number of collaborations related to the ongoing work, including:

- Low cost roll-to-roll manufacturing of biosensors on plastic (J.C. Claussen, Iowa State).
- Food safety agent based risk models (G. Kiker, Univ of Florida) and early warning system development (Muñoz-Carpena, Univ. of Florida) based on nanosensors.
- To expand our work and integrate with wireless sensor networks, we have initiated collaborations with companies specializing in wireless networks for food safety (ScanX, Inc., and AES Controls, Inc.)

Notable results

We have demonstrated the sensing mechanism on silicon microchips, as well as paper and plastic sensors. Currently, we are able to detect *L. monocytogenes* in food matrices at levels as low as 8 CFU/mL within 15 minutes. We are working to improve limit of detection using stimulus response polymers and we have also initiated work to incorporate microfluidic sorting and dielectrophoresis with the aim of improving specificity and transitioning towards high throughput sensing in food processing environments. One of the major factors driving future designs is keeping costs as low as possible while improving sensing resolution and speed.

* For further information about this project link to www.mclamorelab.com or email **emclamor@ufl.edu**

References (10 point font)

Banterle et al (2013) Food Labelled Information : An Empirical Analysis of Consumer Preferences. Int. J. Food System Dynamics 3(2): 156–170

Batz et al (2012) Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. J Food Prot. 75(7): 1278-1291.

CDC (2012) CDC Estimates of Foodborne Illness in the United States. Centers for Desease Control and Prevention, http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html

Engler et al (2012) How far from harmonization are sanitary, phytosanitary and quality-related standards? An exporter's perception approach. Food Policy 37:162–170

FAO (2009). The state of food and agriculture. United Nations Food and Agricultural Organization. Rome, Italy

Koluman A, Dikici A (2013) Antimicrobial resistance of emerging foodborne pathogens: status quo and global trends. Crit Rev Microbiol 39:57–69.

Lê et al (2013) Attitudes toward healthy eating: a mediator of the educational level-diet relationship. Eur J Clin Nutr 67:808–14.

Quested et al (2010) Trends in technology, trade and consumption likely to impact on microbial food safety. Int J Food Microbiol 139 Suppl :S29–42.

RPR (2016). FDA Reportable Food Registry, Pub. L. 110-85

Unnevehr L (2000) Food safety issues and fresh food product exports from LDCs. Agric Econ 23:231–240.

Yue et al (2009) Organic or Local ? Investigating Consumer Preference for Fresh Produce Using a Choice Experiment with Real Economic Incentives. 44:366–371.