Engineering of biosystems for the detection of *Listeria monocytogenes* in foods

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Project Rationale

Pathogenic bacteria cause 90 percent of reported foodborne illnesses. One of these pathogens, *Listeria monocytogenes*, not only causes serious illness, but also can be lethal in infants, people over 60, and immune-compromised individuals. Current methods of detecting *L. monocytogenes* require 15 to 48 hours. Many small food processors and producers do not have in-house capabilities to test for food pathogens and must send out samples for analysis. This adds up to an additional 24 hours. Overall, two to three days typically elapses between when the food is sampled and when the results are available. The time to result (TTR) is problematic since some foods are consumed before test results are available.

Rapid and affordable technologies to detect *L. monocytogenes* cells directly from food and to distinguish living from dead cells are needed. This project addresses the fundamental requirements for developing microchip, bio-based assays that are transportable to the field, useable in a manufacturing environment, and capable of rapidly detecting *L. monocytogenes* at the point of use. Our goals were to achieve microscale detection of *L. monocytogenes* on a real-time or near real-time basis with a TTR of four hours, and to reduce the time of culture steps with rapid cell concentration and recovery based on membrane technology. We are addressing the development and validation of such a microchip system that combines bioseparations technology—for rapid concentration and recovery of microbial cells, and bionanotechnology—to construct systems capable of interrogating fluids for pathogens.

Project Objectives

- Develop a system for rapid cell concentration and recovery. Improve membrane chemistry and methodology for handling food samples high in fat and complex molecules presented by blended hotdog, hamburger, vegetables, milk, and meat, and decrease the volume in which the cells are captured by selecting or constructing the appropriate membrane design, then combining with other bioseparation techniques. For validation, use GFP engineered cells, as well as non-modified cells, in mixtures of pathogenic and non-pathogenic microorganisms.
- Correlate media composition to changes in growth characteristics and metabolism of *L. monocytogenes* cells, and develop media that enhance the response of pathogenic cells to detection methods. A complimentary objective was to improve low conductivity media for the enhanced capture and detection of stressed cells by antibodies and other bioreceptors.
- Combine antibody-based capture and growth detection for the biochip. Design and microfabricate integrated devices to combine ATP, pH, and/or direct nucleic acid and antibody-based detection on-chip using multi-channel, multi-functional designs. The goal was to obtain biochips that sense multiple parameters simultaneously and improve dielectrophoresis (DEP)-based selective capture of *L. monocytogenes* and other pathogens in mixtures of cells and to test sensitivity and selectivity of capture.

Project Highlights

We integrated the multiple functions needed for the development and deployment of microfluidic biochips for detecting bacterial pathogens. We integrated antibody-based capture of bacterial cells enhanced by dielectrophoretic forces, bacterial culture and electrical detection of bacterial growth, and PCR-based detection of *L. monocytogenes*—all on chip. A significant accomplishment was the development of label-free electrically amplified PCR products. We showed that impedance can be used to detect the presence of DNA molecules in a solution without any labels directly from the actual PCR solution. This could lead to user-friendly miniaturized PCR detection systems that do not need optical labels or optical detectors.

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