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

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Project Rationale

Pathogenic bacteria cause 90% of reported foodborne illnesses. *Listeria monocytogenes* has emerged as one of the most important food pathogens, having a "zero tolerance" in ready-to eat processed (lunch) meats and dairy foods. This bacterium not only causes serious illness but also is lethal in infants, people over 60, and immune-compromised individuals.

Current methods to detect this bacterium rely upon enrichment to increase the number of bacteria present in a sample. The food or food extract is incubated in special growth media for 12 to 24 hours and the resulting culture is tested for *L. monocytogenes* using procedures that require an additional 3 to 24 hours. The food industry includes many small food processors and producers that do not have in-house microbiological laboratories for the purpose of testing for food pathogens. Therefore, many companies send out samples for analysis. This adds up to another 24 hours to the time that elapses between when the food is sampled and the bacterium, if present, is detected. An overall time of 2 to 3 days typically elapses from when the food is sampled and the test results are available. The elapsed time, referred to as "time to result" or TTR, is problematic since some foods are consumed before test results would be available.

Rapid and affordable technologies to detect low numbers of *L. monocytogenes* cells directly from food, and which distinguish living from dead cells, are needed. This multi-disciplinary, multi-departmental research project is addressing the fundamental engineering and science required for development of microchip, bio-based assays that are transportable to the field, useable in a manufacturing plant environment, and capable of rapidly detecting *L. monocytogenes* at the point of use. This research has the goals of (a) microscale detection of *Listeria monocytogenes* on a real-time or near real-time basis with a time-to-result of 4 hours, and, (b) reducing the time of culture steps with rapid cell concentration and recovery based on membrane technology.

Our multidisciplinary research team is addressing the development, engineering and validation of such a microchip system that combines bioseparations technology for rapid concentration with recovery of microbial cells and bionanotechnology to construct systems capable of interrogating fluids for pathogens. Our approach is resulting in a technology platform capable of detecting other types of foodborne and medically relevant pathogens, even the focus of the research is on rapid detection of *Listeria monocytogenes* by a combination of technologies that will ultimately give a time to result of hours.

Project Objectives

Biochips are needed that are affordable, capable of rapid detection of food pathogens, and easy to use by small food processors as well as major food companies. The goals and associated milestones of our research are:

- Rapid concentration and recovery of microorganisms from food samples for subsequent interrogation of pathogens.
- Sampling and conditioning fluids containing the cells while maintaining their information content (i.e., the molecules or cells that represent possible targets of the chip).
- Transporting sample fluids on and/or off the chip so that target microbes are captured and retained so that they can be probed for presence of pathogens.
- Interfacing biological molecules (i.e., biomolecules) with electronic components.
- Electronically detecting and amplifying biomolecular interactions between target and the biomolecules that form the biorecognition components of the chip.
- Achieving in vitro biospecificity for the target molecule.
- Interfacing biochip systems with electronic reading devices.

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Project Highlights

The integration of cell concentration and recovery, sample introduction to the biochip, parallel detection of pH and conductivity, and improvements in specificity and sensitivity have a common basis in rapid detection and quantitation of small differences in conductivity or changes in pH. This difference is maximized using a low conductivity buffer that will support cell viability and growth. The low conductivity growth medium (LCGM) that includes compounds such as tryptone, yeast extract, glucose, BSA and other constituents to yield a low conductivity of 1.2 mS. Proteins expressed by LCGM were identified to include superoxide dismentase, thiol peroxidase, and unknown lipoproteins. These over-expressed proteins could be used as target proteins for development of new antibodies, and for direct or indirect measurement on-chip, thereby enhancing sensitivity. Overall, the validation of LCGM over the last year resulted in the practical impact of increasing sensitivity of on-chip detection of *L. monocytogenes*.

Our interdisciplinary research approach has enabled us to learn about the biology of interactions between microorganisms and nutrient-rich foods; mechanisms of their transport and capture in microfluidic systems; expression of biomarkers under environmental stress (i.e., conditions during food handling and/or sampling); and characteristics that impact their viability under environmental stress. The stress that the microorganisms may experience is related to changes in micro-environments during the course of sampling and rapid detection protocols.

Initial runs with milk and vegetables have been carried out to begin examining rapid recovery of microorganisms internal to the biological tissue as found in various types of foods. Ultimately, the fundamental knowledge gained will apply to a broad range of samples (water, air, packages, animals, and people) for which fluids are probed to rapidly assess the level of risk by interrogating these samples for biomarkers that indicate biological or chemical toxins. Such biomarkers may include proteins, peptides, low molecular weight metabolites, chemical or bio-toxins, and lipids, as well as nucleic acids. This is being studied in the context of on-chip pathogen detection and integration of preprocessing steps. The rapid concentration and recovery of the microorganisms has advanced with the use of hollow-fiber membranes that are able to process extracts from foods that contain the microorganisms, and are amenable to use in a hands-off system that ultimately will lead to an automated instrument. A team approach is required by the complex interactions of the various components – both biological and electrical – of the detection system. These include dielectrophoresis and antibody-mediated selective capture of microorganisms in microfluidic biochips. The multidisciplinary cooperation among the team has enabled significant progress to be made in the integration of the various subsystems.

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