



Rapid, quantitative, and reusable immunosensors for bacteria detection on a microfluidic platform

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Project Rationale

Portable, rapid, and sensitive biosensors for food safety applications enable point-of-care contamination detection and immediate interpretation of the results. In our research project, we proposed to develop an integrated biosensor system on a microfluidic chip for detecting bacteria based on immunoassays. The device will offer a sensitivity of 10^2 to 10^3 bacteria cell detection and an assay time of fewer than 20 minutes for a single test. Our system will yield quantitative data for estimating the number of the target bacterium in a food sample. The microfluidic system will consist of individual devices for cell lysis, lysate purification, and immunoassays. In principle, the tool will be effective for any bacterium or strain given the availability of a suitable intracellular antigen-antibody pair. In this project, we will demonstrate the concept using an intracellular antigen, alcohol acetaldehyde dehydrogenase (Aad), and its antibody MAb-H7 to detect *Listeria monocytogenes*. In order to concentrate *L. monocytogenes* cells from food samples, we will fabricate magnetic nanobars with different sizes and geometries and develop protocols for immobilizing antibodies specific to *L. monocytogenes* on the surface.

A portable, reusable, and low-cost device would be useful for point-of-care analysis in the food manufacturing industry. Conducting bacteria detection tests within food manufacturing laboratories would dramatically decrease the turnaround time for the results and avoid potential contamination and changes in the bacteria during transit. Conventional analytical methods require bulky, expensive equipment that are often cost-prohibitive for food manufacturing laboratories. With our lab-on-a-chip approach, sophisticated functions of a biological laboratory can be miniaturized on a microchip, enabling any minimally equipped laboratory with the ability to perform bacteria detection tests. This technology can significantly benefit the food industry by enhancing the laboratory-testing capabilities of food

manufacturers and food testing laboratories as well as field-testing activities of governmental agencies.

Project Objectives

- Fabricate magnetic nanobars with different sizes and geometries and develop protocols for immobilizing antibodies specific to *L. monocytogenes* on the surface. The amount of bacterial cells bound to the surface will be characterized under different conditions.
- Develop an electrophoresis-based immunoassay coupled with laser-induced fluorescence on a microfluidic chip. We will use this tool to quantitatively detect *L. monocytogenes* based on cell lysate via the interaction between alcohol acetaldehyde dehydrogenase (Aad) and its monoclonal antibody (MAb-H7).
- Demonstrate a prototype-integrated microfluidic system which incorporates different steps such as manipulation of magnetic nanobars, cell lysis, lysate purification, and immunoassay.

Project Highlights

We integrated the concentration, lysis, and competitive immunoassay for detecting *L. monocytogenes* on a microfluidic chip. A packed bed of microbeads, with the bead surface coated with monoclonal antibody (MAb-H7) that is specific to the antigen *Listeria* adhesion protein (LAP), was formed in a microfluidic channel to physically trap bacterial cells. Electrical lysis was then used to release intracellular materials from the trapped cells. Fluorescein isothiocyanate (FITC)-labeled LAP was flowed through the microbead bed to bind to unreacted antibody sites and reveal whether LAP was present in the bacterial sample. By integrating all these functions onto one simple portable chip, we can produce a biosensor system that is both highly efficient and inexpensive.

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