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

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Overview

Immunomagnetic separation (based on nanobars) of bacterial cells from food samples

Cell lysis
Electrophoresis-based immunoassays based on intracellular antigens

Attributes of our design

- Magnetic nanobars: larger magnetic force for separation and less clogging in microchannels compared to microspheres
- Rapid: 30 min-1 h for off-chip cell separation and <10 minutes for on-chip detection
- Quantitative: laser induced fluorescence detection allows estimation of the number of pathogenic cells
- Reusable: the same microchip for different pathogens for multiple tests (no immobilization for immunoassays)

Fabrication of magnetostrictive nanobars

Process

I. Porous membrane
II. Deposit bottom Au-electrode
III. E-C deposit FeB (Bath C)
IV. Dissolve Membrane

The shape and morphology of nanobars

- Nanobars: diameters from 50 to 200 nm
- The structure and morphology as required

Freestanding nanobars

Freestanding magnetostrictive nanobars were released from substrate
Electrical lysis of cells

Electrical lysis of bacterial cells

GFP expressing L. monocytogenes

Expression of GFP (GFP was placed under the control of aad promoter) in L. monocytogenes

* This strain can be used to monitor cell lysis and AAD release on chip.

Distribution patterns of MAb-H7 specific AAD in L. monocytogenes cells

Electrophoresis-based immunoassays

Western blot analysis of AAD expression in WT and mutant strains

Experimental setup

Layout of the electrophoresis chip

Table:

| Field Strength (V/cm) | Collected number of fluorescent bacteria
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<td>2500</td>
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The movement of GFP expressing E. coli cells with 1500 V/cm in the narrow section

When F2 is higher than 2000 V/cm, the fluorescence from cells is no longer seen at the exit of the narrow section.

Experimental setup
On-chip detection of recombinant AAD

![Graphs showing fluorescent intensity against migration time for different concentrations of Antibody and Aad.]

Compact systems for biochip reading

![Image of a compact biochip reader.]

Future work

- Immobilization of antibody on magnetic nanobars for IMS
- Testing of different cellular antigens (InlA, InlB, ActA) in *L. monocytogenes* which will improve the specificity (current assay detects *Listeria* species only)
- Testing of electrophoresis-based immunoassays based on *L. monocytogenes* lysate
- Integration of cell lysis and electrophoresis-based immunoassays