Microfluidic immunomagnetic separation for enhanced bacterial detection

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Abstract

A combined lab-on-a-chip approach combining immunomagnetic separation (IMS) and flow cytometry was developed for the enrichment and detection of salmonella contamination in food samples. Immunomagnetic beads were immobilized in chips consisting of long fractal meanders while contaminated samples were flowed over them. After incubation the beads can be released for detection into the flow-cytometry chip. Immunomagnetic beads were prepared by using anti-Salmonella antibodies and magnetic beads (2.8µm). Both the synthesized and commercially available anti-Salmonella beads were used to capture Salmonella from samples artificially contaminated with a concentration range of 10² to 10⁷ cells/ml and the captured Salmonella were then detected and quantified flow cytometrically after vital staining.

The results showed that on-chip IMS could effectively discriminate a bacterial contamination of 10⁴/ml from control (0.22 µm sterile filtered buffer) with 65-85% recovery of magnetic beads. The parameters of the IMS and staining process (incubation time, temperature and buffering conditions) were optimized. Optimum interaction time and temperature was 20 minutes and 22°C (room temperature) respectively to obtain maximum capturing efficiency. The effects of channel volume, path length and number of bends of microfluidic chip on IMS efficiency were also determined.

Experimental

Immunomagnetic beads (2.8 µm diameter) were coated with anti-Salmonella antibody, washed twice and resuspended in PBS buffer. For conventional IMS experiments, 1 ml samples were prepared with a range of Salmonella bacteria from 10² to 10⁷, and they were mixed with 6 x 10⁶ of the prepared beads. After incubation (20 min. at room temp) a Dynal Invitrogen bead separator was used to perform 2 sequential washing steps with resuspension in buffer. Finally fluorescent staining was performed using Carboxyfluorescein Diacetate (CFDA) (incubated 20 min. at 37°C) and flow cytometry analysis performed in a Cytomics FC 500 instrument.

For the on-chip IMS experiments, a number of beads (3 x 10⁵) were instead captured inside the microfluidic chip using the permanent magnet fixture, and the different sample concentrations introduced. Staining procedure and flow cytometry was the same as for the conventional IMS.

References


Figure 1. Left: Model of the magnetic field generated by placing 4 15x15 mm, rare-earth permanent magnets in a rectangular matrix. Inset rectangle shows intended placement of microfluidic chip, and shows how an artificial sample would be laid perpendicular to the chip. Right: Illustration of the mechanical design made to hold 4 magnets in the intended position, an easy attachment to the microfluidic chip holder, which connects to the existing micro-fluidic setup (based on Thorlabs standard 20x30 mm optics rack system).

Figure 2: Different space-filling Peano curve designs have been tested, varying channel crosssection dimension, total channel length and volume, number of turns etc. in order to determine the optimal conditions for maximum IMS bead/sample bacteria interactions. Top left to right – effect of doubling total length as well as number of turns investigated. Top to bottom left – effect of doubling total length but keeping number of turns unchanged determined. In bottom right the total length as well as number of turns is increased, and an on-chip filter added to remove large dust particles on sample inlet.

Figure 3. Image series showing from top the microfluidic setup with an IMS chip mounted in the white holder through to how the magnet assembly is easily placed on the chip. The IMS chip is connected to the next chip (flow cytometry chip) in the black holder above.

Figure 4: Illustration of the process developed for producing PDMS microfluidic chips – from mask designs on the left, through a photolithographic process to create mold masters towards moulding and assembling of the finished chip.

Figure 5: Flow cytometry results from Cytofence FC 500 flow cytometer, comparing different cell concentrations to reference samples using respectively traditional IMS and the new IMS microfluidic chips.

Figure 6: Distribution of spheres used for testing the IMS efficiency. Percentage of spheres captured by the IMS system.

Figure 7: NMR spin echo experiments with different concentrations of magnetic beads.

Figure 8: Schematic diagram of the microfluidic IMS chip.

Figure 9: Illustration of the process developed for producing PDMS microfluidic chips – from mask designs on the left, through a photolithographic process to create mold masters towards moulding and assembling of the finished chip.

Figure 10: Flow cytometry results analyzed and presented using Cytomics CXP Analysis software.

Figure 11: Distribution of spheres used for testing the IMS efficiency. Percentage of spheres captured by the IMS system.