Integrated Microchip Sensors for Detecting *Escherichia coli* O157:H7 and *Salmonella enterica Based on TaqMan Assays*

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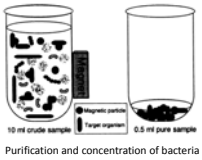
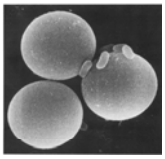
Microfluidic Sensor based on Manipulation of Magnetic Beads

- Integration of multiple steps for PCR based detection
  - Bacterial cell capture by immunomagnetic separation (off-chip)
  - Cell-bead complex concentration by magnetic field
  - Electric lysis of cells and release of plasmid DNA
  - Adsorption of plasmid DNA on packed bed of magnetic beads
  - TaqMan PCR assay (off-chip)

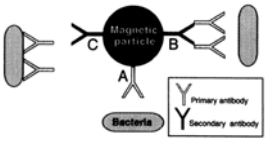
Immunomagnetic Separation (IMS) for Bacteria Separation from Food Matrix



Purification and concentration of bacteria



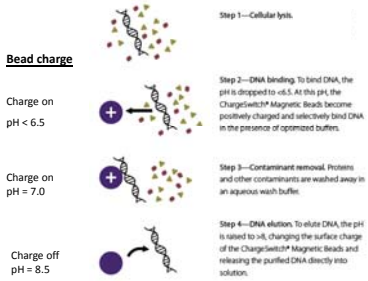
Scanning electron microscopy image of bacteria bound to Dynabeads M450 coated with antibody



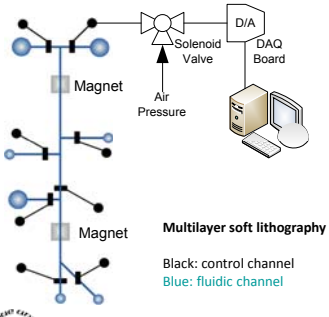
Dynabeads ® anti-*Salmonella*

Olsvik O., Popovic T., Skjerve E., Cudjoe K.S., Hornes E., Ugelstad J., Uhlén M. **Clinical Microbiology Review** 7 (1994) 43-54.

ChargeSwitch® Magnetic Beads for Plasmid DNA Collection

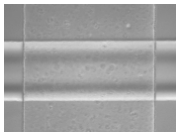


Microchip Design



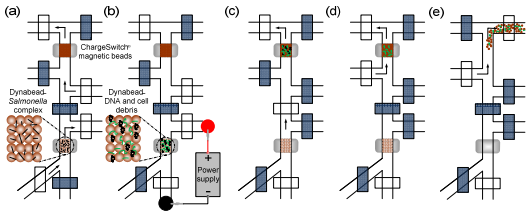
Multilayer soft lithography

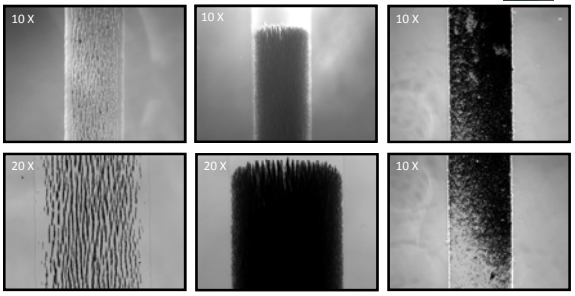
Black: control channel
Blue: fluidic channel



Fully closed valve (Valve closing for rounded channel)

On-chip Cell Concentration, Lysis and DNA Purification



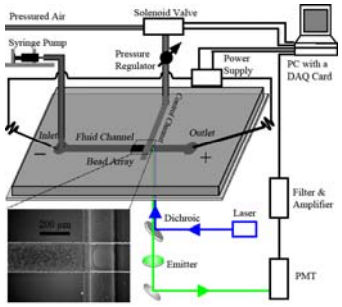


Flowing magnetic beads under magnetic field

Magnetic bead plug

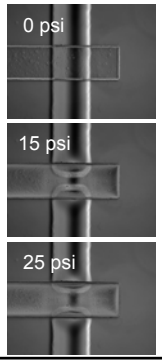
Magnetic beads without magnetic field

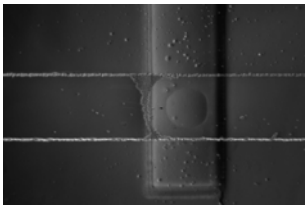
Microscale bead array for capturing and lysing bacterial cells in a microfluidic device



Bao et al. ***Applied Physics Letters*** 92 (2008) 214103.

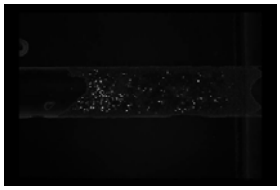
Multilayer valve and capture of microscale beads (d~5 μm)





The speed of the video is 14X of the real process

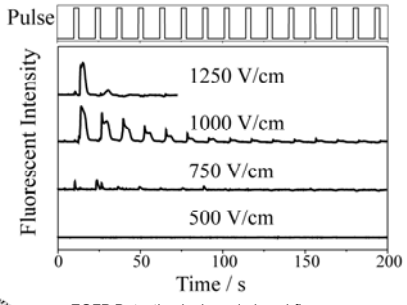
The capture process



E. coli cells (10^7/ml) with 5 μm bead matrix

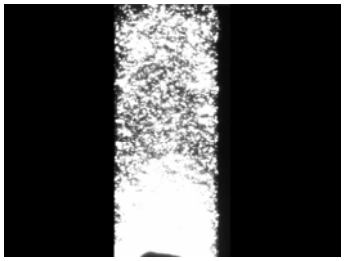
E. coli cells (10^9/ml) with 5 μm bead matrix

Electrical lysis of bacterial cells and release of the intracellular proteins



EGFP Detection by laser-induced fluorescence in the downstream of the bead array

The electrical lysis

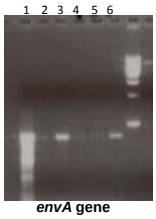


Electrical pulses: 2000 V/cm, 2s duration

2 pulses with a 18 s interval in between

The video is 1.4 X faster than real time

Preliminary Results



***envA* gene**

PCR conditions:
initially at 94 °C for 1 min, 29 cycles at 94 °C for 1 min, at 50 °C for 1 min, at 72 °C for 1 min, and a final extension at 72 °C for 10 min

Primer:
Farward:
CGGTGGTTTTAAGCGTACTCTT
Reverse :
CGAATATGCTCCACAAGGTTA

Lane 1: Positive control, cells were lysed by heathing for 10 min at 90 °C, and DNA were not purified. ~10^5 cells

Lane 2: 1500 V/cm (10 s interval and 1 s pulse for 3 min), ~10^6 cells

Lane 3: 1200 V/cm (10 s interval and 1 s pulse for 3 min), ~10^6 cells

Lane 4: 1000 V/cm (10 s interval and 1 s pulse for 3 min), ~10^4 cells

Lane 5: 1000 V/cm (10 s interval and 1 s pulse for 3 min), ~10^4 cells, from washing of the chargeswitch beads

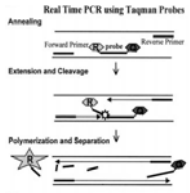
Lane 6: 1000 V/cm (10 s interval and 1 s pulse for 3 min), ~10^7 cells

Current Challenges and Future Work

Problems:
1.Unknown cell number in each experimental run.
2.Low sensitivity.
3.DNA desorption from chargeswitch beads.

Future work:
1.Apply cells expressing fluorescent protein (e.g. GFP) for enumeration of cells.
2.Pinpoint the bottleneck for improving sensitivity among several effects: completeness of electric lysis release, DNA capture efficiency, DNA adsorption on IMS beads, PCR condition
3.Apply RT-PCR.
4.Reduce the flow rate of wash buffer, and increase the density of ChargeSwitch® magnetic beads.

Real-Time PCR Assay



1. Probes and primers anneal to target sequence. Taqman probes have two covalently linked fluorescent dyes: a reporter (R) and a quencher (Q). On the probe, the reporter dye emission is quenched.
2. During each extension cycle, the 5'→3' exonuclease activity of *Taq* DNA polymerase cleaves the reporter dye from the probe.
3. Once separated from the quencher, the reporter dye emits its characteristic fluorescence, which is measured in every cycle by a detector.

TaqMan® Pathogen Detection Kits (Applied Biosystems)
Primers and Taqman Probes: Applied Biosystems Primer Express s

Conclusions

- Magnetic beads are manipulated by a magnet in a microfluidic channel to form a closely packed bed
- Plasmid DNA can be released from *Salmonella* cells by electric lysis
- PCR is conducted based on extracted DNA from bacteria

Acknowledgements

- Dr. Ning Bao, Tao Geng, Dr. Kirshna Mishra
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