An LSPR sensor coupled with magnetic nanobeads-based immunoseparation for rapid and sensitive detection of *E. coli* O157:H7

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Abstract

Escherichia coli O157:H7 is one of the most commonly identified foodborne pathogens for humans and animals, which pose a threat to human health and cause substantial economic loss to society. The rapid and sensitive detection of foodborne pathogens is critical to ensure food safety.

The objective of the present study was to develop a localized surface plasmon resonance (LSPR) sensor coupled with magnetic nanobeads-based immunoseparation (MNBs-IS) for rapid and sensitive detection of *E. coli* O157:H7 in foods.

Biotinylated anti-*E. coli* O157:H7 antibodies were immobilized on streptavidin pre-coated MNBs (100 nm) surface to specifically capture and isolate target bacteria from food matrix. The cleaned LSPR sensor chip was functionalized with 16-mercaptohexadecanoic acid, and then was activated with carbodiimide hydrochloride and *N*-hydroxysuccinimide to covalently bond with anti-*E. coli* O157:H7 antibody. After being blocked with polyethylene glycol, the sensor chip was ready for detection. In tests, 100 µl of the separated MNBs-*E. coli* complexes were pumped into the LSPR chip and measured using a LSPR analyzer.

The results showed that the LSPR/MNB-IS sensor could shorten the detection time down to 4 min, with a flow rate of 20 µl/min. The detection range of *E. coli* O157:H7 was 10^2 to 10^7 CFU/ml, with a detection limit as low as 36 cells in a sample of 100 µl. The MNBs used in this study were served not only in sample pretreatment, but also in amplification of the detection signal. When *E. coli* pure culture with the concentration below 10^5 CFU/ml was tested without MNBs, no binding signal was observed, which confirmed the signal amplification by MNBs. No interference was observed with non-target bacteria.

The developed LSPR/MNB-IS sensor is potentially a rapid, specific and simple approach for detection of *E. coli* O157:H7 at low concentrations in foods.

Introduction

Escherichia coli O157:H7 is one of the most commonly identified foodborne pathogens, which poses a great threat to human health and may cause substantial economic loss to society. The rapid and sensitive detection of foodborne pathogens is critical to ensure food safety, however, has been a well know challenging project in biological applications.

The development of optical transducers using surface plasmon resonance provides a powerful tool for analyte detection, yet highly rapid and sensitive detection of bacteria is not well studied and achieved.

Objective

To develop a LSPR sensor coupled with MNBs-IS for rapid, specific and sensitive detection of *E. coli* O157:H7 in food samples.

Materials & Methods

Material:

- Reagents:
  - 16-mercaptohexadecanoic acid (MHDA), 20 mM
  - *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC)/ N-hydroxysuccinimide (NHS), 70 mM/30 mM 1:1
  - Streptavidin, 1 mg/mL
  - Biotinylated rabbit anti-*E. coli* antibody, 1 mg/mL
  - Bovine serum albumin (BSA), 3%
  - Poly(ethylene glycol) methyl ether thiol (PEG), 0.1 mg/mL
- Magnetic beads:
  - Super magnetic streptavidin beads, 1 mg/mL, 100 nm diameter
- Bacteria:
  - *Escherichia coli* O157:H7 (ATCC 43888)

Apparatus:

- Nicoya OpenSPR

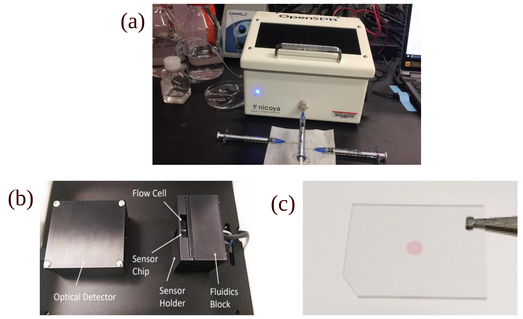


Figure 1. Nicoya OpenSPR (a) Whole system; (b) Internal structure; (c) Sensor chip

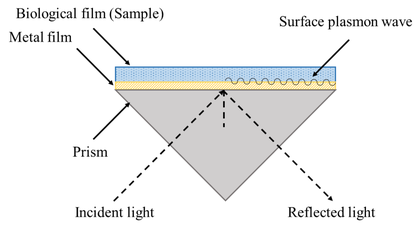


Figure 2. Principles of LSPR

Methods:

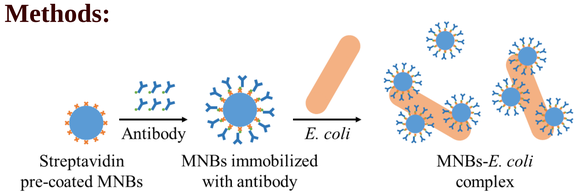


Figure 3. Magnetic nanobeads-based immunoseparation

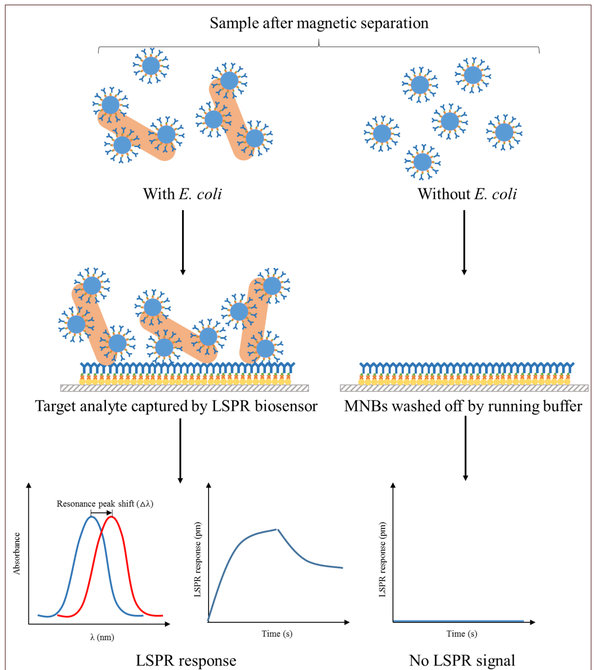
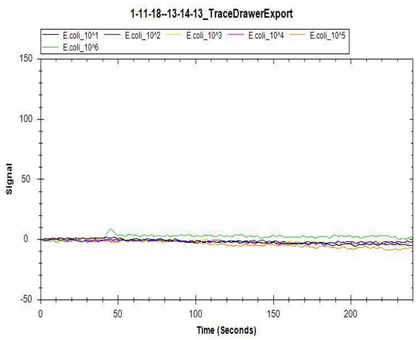


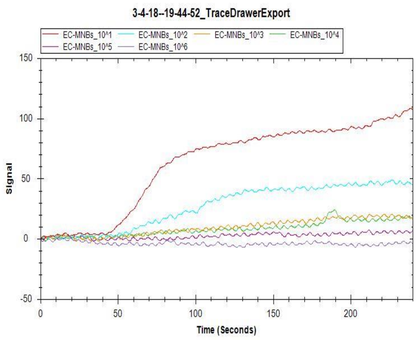
Figure 4. Detection of *E. coli* with LSPR biosensor

Preliminary Results



E. coli (CFU/mL)	LSPR signal (pm)
10^1	-5
10^2	-2
10^3	-7
10^4	-3
10^5	-3
10^6	3

Figure 5. LSPR signal of *E. coli* (10^1 to 10^6 CFU/mL)



E.coli-MNBs (CFU/mL)	LSPR signal (pm)
10^1	-11
10^2	1
10^3	10
10^4	8
10^5	21
10^6	97

Figure 6. LSPR signal of MNBs-*E. coli* (10^1 to 10^6 CFU/mL)

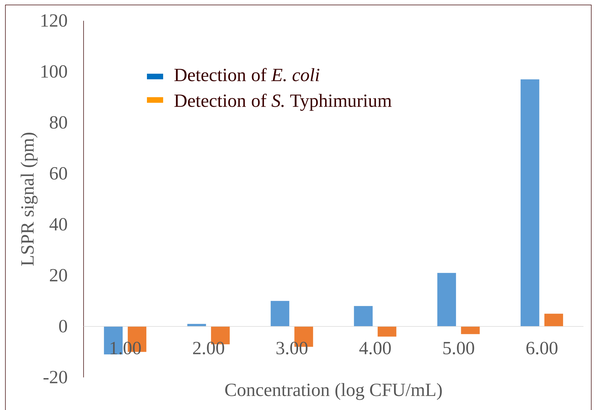


Figure 7. Specificity test with *S.* Typhimurium

Conclusions

- The results showed that the LSPR/MNB-IS sensor could shorten the detection time down to 4 min, with a flow rate of 20 µL/min.
- The detection range of *E. coli* O157:H7 was 10^2 to 10^7 CFU/mL, with a detection limit as low as 36 cells in a sample of 100 µL.
- The sensitivity of this biosensing method is 5.8 pm/log CFU mL^{-1}.
- The specificity was approved with *S.* Typhimurium.
- The MNBs used in this study were served not only in sample pretreatment, but also in amplification of the detection signal.
- The developed LSPR/MNB-IS sensor is potentially a rapid, specific and sensitive approach for detection of *E. coli* O157:H7 in foods.

Acknowledgments

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References

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