Biosensor Integrated Spectroscopy for Pathogen Capture & Detection

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**Objectives**

- Develop fingerprint library using Raman and FTIR for key foodborne strains
  - Investigate microbial components
  - Dead vs live; Treatment conditions
- Advance portable infrared biosensor
  - Optimize biosensor platform
  - Appropriate sampling methods and Testing
  - Nanoscale biosensors

**Pathogens tested**

- *E. coli*  
  - *E. coli* O157:H7 (Acc No: 5.2262, 99.0874, 0.1292, 99.0894, 0.0027, 0.1288, 0.1304, 7.3853, 7.3860)
- *Salmonella*
  - *S. typhimurium, S. enteritidis*
- *Listeria*
  - *L. innocua, L. monocytogenes*
- *Shigella flexneri, Staphylococcus aureus*

**FTIR vs Raman**

Raman spectrum (red) is more highly resolved than the FTIR spectrum (purple).

**Raman and FTIR discrimination**

Differentiation of five different species of pathogenic bacteria based on the canonical variates

**Strain level discrimination by Raman and FTIR**

Discrimination of five different *E. coli* O157:H7 strains obtained from different sources.
Surface Enhanced Raman Spectroscopy using Silver Nanospheres


A) SERS spectra of S. aureus on the as-prepared AgNSs with different concentrations from a, 10^6; b, 10^5; c, 10^4; d, 10^3; e, 10^2 and f, 10 cfu/mL

B) Comparison of SERS spectra of E. Coli O157:H7R52262 (a), S. Typhimurium (b), and S. aureus (c) at 785 nm excitation [10^2 cfu/mL]

CVA for species and strain level differentiation

Gold nanosensors detects multiple pathogens

1. Dual Platform: UV-Visible & Raman spectroscopic techniques for detection
2. Dual Particle Probes: Gold nanoparticles & Ag-Au Core-Shell nanoparticles
3. Cross-Platform: Antibody/Aptamer for capturing specific pathogens
4. High Sensitivity: As few as 100 CFU/ml
5. Rapid & Accurate: Assay takes less than 30 minutes.

- Detection by UV-vis used Localized Surface Plasmon Resonance properties of gold nanoparticles: (i) absorption peak around 520 – 530 nm, (ii) peak red-shifts upon attachment of targets
- Detection by Raman uses Raman labels

Basic optical properties of nanoparticles

The red axis represents that of spherical gold nanoparticles. The Localized Plasmon Resonance peak appears at about 520 – 530 nm.

The green axis represents rod shaped gold nanoparticles. Has a transverse absorbance similar to Spherical particles and longitudinal absorbance (> 600 nm) depends on aspect ratio.

UV-Vis and Raman Spectroscopy integrated biosensors for multiple pathogen detection

Ravindranath et al. 2009. Multiplex detection methodologies and cross platform techniques in pathogen sensor development. (Manuscript in Preparation)
Sensor 1: UV-Vis based Multiplex Detection

4- Biofunctionalized probes before and after interaction with pathogens

Shift in the peak of biofunctionalized gold nanoparticles upon interaction with specific pathogens

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Multiple pathogens detection, separation and photothermal ablation

UV-vis absorbance spectra after addition of a mixture of E. coli and S. typhimurium to anti-E. coli and S. typhimurium antibody-conjugated amine modified gold nanorods of aspect ratios 2.0 and 3.2, respectively. The concentrations of E. coli and S. typhimurium were 1.0 to 10^6 cfu/mL.


Sensor 2: Raman based Multiplex Detection

S. typhimurium
E. coli O157:H7
S. aureus
L. monocytogenes

Unique Raman label specific to each of the pathogens

Research Summary Phase I

- Fingerprints of pathogens using Raman and FTIR
    • Enhanced fingerprint using Silver spheres – LOD of 10 cfu/ml
- Multiplex detection using LSPR properties of gold nanoparticles – LOD of 10 cfu/ml
- Multiplex detection with a separation step

Sensor 1: Mid-IR Spectroscopic Biosensor

Spectral Resolution comparison

Comparison between Spectrometers

Cost: $9000 vs $125000 (Benchtop)
Wt: 3.5lbs; Operation: 15°C - 60°C

Trapping of E. coli using Magnetic Nanoparticles and FTIR detection

Biosensor concept validation in a Portable system

Rapid formation of Nanoparticle mediated bacterial clusters – an indirect signal enhancement

Portable Mid-IR Biosensor for pathogen detection

Separation and detection of multiple pathogens using Magnetic SERS probes

Magnetic Nanoprobe concept:

Magnetic nanoparticles are coated by silica to provide better biocompatibility and surface properties

SERS Nanoprobes: to detect bacteria with high sensitivity

Simply change the Raman Reporters (RR) and antibody, multiple bacterial identification can be achieved.
Quantifying receptor characteristics using single molecule spectroscopy

Separation and detection of pathogens in a food matrix

Detection in Peanut butter by SERS

Blind experiment for detection in peanut butter. The spectrum indicates that the pathogen is S. typhimurium.

SERS spectra for S. aureus and S. typhimurium in peanut butter (a. 0 cfu/mL, b. 10^2 cfu/mL, c. 10^3 cfu/mL, d. 10^4 cfu/mL, e. 10^5 cfu)

Single Molecule Quantification

Aptamers for S. typhimurium

Research Summary Phase II

• Portable mid-infrared biosensor assay conceptualized and demonstrated (2% milk, milk, spinach)
• Multiplex detection with a separation step using uv-vis platform
• Multiplex detection using Raman (Peanut butter)
• Single molecule quantification

Overall Deliverables (June 2007 – March 2009)

Publications

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