Paper-based Microfluidic Sensor for Detection of Pathogenic Enzymes Chemotaxis

Aim of the work:

Urease is a nickel-containing enzyme that catalyses the hydrolysis of urea into ammonia and carbon dioxide. This enzyme occurs in such different organisms as bacteria, algae, fungi and higher plants. Its primary function is allowing the organism to use urea as a nitrogen source. In plants, urease is involved in systemic nitrogen transport pathways, and is thought to act as a toxic defence protein. In humans, bacterial ureases are important virulence factors in a number of diseases of the urinary tract and gastroduodenal region, including cancer. Our aim is to develop a fundamentally new approach like design and development of a paper-based microfluidic sensor for rapid isolation and concentration of such pathogenic enzyme using molecular chemotaxis for facilitating early disease diagnosis and surveillance.

Plan of the work:

*(a) COMSOL simulation **(at IITGn)***
*(b) Design and Development of low-cost diagnostic platform **(at IITGn)***
*(c) Substrate and enzyme immobilization and electrical signal measurement (at **IITG**)*
*(d) Data analysis (at **IITG** and **IITGn**)*

Work plan in IITG: *Substrate and enzyme immobilization and electrical signal measurement*

A. **Device Fabrication:** The microfluidic device comprises a sample zone, a hydrophilic channel, a test zone and two conductive Al electrodes. Wax patterning technique was used to create hydrophilic channels on the surface of a Whatman grade 1 filter paper. Pre-conceptualized designs of the hydrophilic channel are printed on the paper substrate while the electrodes are deposited using glove box. The filter paper rubbed with wax surrounding the channel region is heated to cause the wax to reflow along the thickness of the paper that selectively creates hydrophobic barriers surrounding the hydrophilic region. The electrical signal of enzyme-substrate with the immobilized enzyme in the test zone is measured by using Keithley 4200 semiconductor parameter analyser.

B. Substrate used: Urea
Enzyme used: Urease
Buffer: (1) PBS (pH=7.2) of 100 mM and (2) Acetate buffer (pH=5.2) of 100 mM

1. *Pathogenic Enzymes Chemotaxis detection by varying the conc. of Urea in PBS Buffer:*

   Urea Concentration: 10 M, 1 M, 1 mM, 1 µM, 1 nM, 1 pM (in DI water)
   Urease Concentration: 25 mg/ml (in **PBS** 100 mM)

2. *Pathogenic Enzymes Chemotaxis detection by varying the conc. of Urea in Acetate Buffer:*

   Urea Concentration: 100 M, 10 M, 1 M, 1 mM, 1 µM, 1 nM, 1 pM (in DI water)
   Urease Concentration: 25 mg/ml (in **Acetate Buffer** 100 mM)