Title:	Aptamer functionalized nanostructured biosensing platforms for enteric pathogenic bacteria
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Abstract: Out of the vast myriad of bacterial species that colonize the gastrointestinal tract, Escherichia coli has been a persistent member of the mammalian gut microflora where a majority of its strains maintain a commensal or mutualistic relationship with the human host 1 . However, its disease-causing variants (pathogens) genetically encoded with specific virulence factors also exist 2,3 . Pathogenic E. coli has been reported as a significant etiological agent of bacterial gastroenteritis with an infectious dose as low as ~100 cells 4,5 . These are responsible for chronic or persistent diarrhea, with certain pathotypes producing enterotoxins responsible for the hemolytic uremic syndrome as sequela 6–8 . Conventional 'gold standard' microbiological tools require 2-3 days for identification, while the current diagnostic approaches majorly reliant on antibody-based assays or mass spectrometry, utilize costly reagents and require specific instrumentations for estimation 9,1011 . Thus, the challenge lies in developing sensitive, rapid, non-culture dependent, and cost-effective methodologies for the sensing of enteric pathogenic bacteria, which are required for timely monitoring in food, clinical, and municipal sectors. Integrated biosensing platforms with specific bio-receptor (nucleic acid aptamers) and sensitive transducer (nanostructured materials), providing near real-time information on the presence of enteric targets, are proposed in this doctoral thesis. Nanostructures of various dimensions based on carbon, metal-carbon nanohybrids, and more recently, its 2D analogs transition metal chalcogenides, harboring fascinating electronic, optoelectronic, and physical qualities such as abundant surface charges, high surface-to-volume ratio, and easy functionalization abilities were synthesized indigenously via various top-down and bottom-up approaches and utilized as transducers in the biosensing platforms. Simultaneously, for the bio-receptor component, deoxynucleic acid (DNA) aptamers (single-stranded oligomers with high binding affinity with the specific target) were chosen, providing low cross-reactivity than conventionally available counterparts 12,13 . The studies reported in this thesis majorly gravitate around biosensing pathogenic E. coli (serotypes O78:H11 and O157:H7) with aptamer-based nanostructured sensing technology as the critical line of detection approach. We devised a three-way approach for aptamer generation stemming from alternative enhanced binding affinities for the target in various environments, which was based on the hypothesis that the presence of both whole-cell or its components is possible for monitoring the bacterial targets. Therefore, multiple respective aptamers were screened against the avirulent strains of whole-cell E. coli (enterotoxigenic serotype O78:H11 and enterohemorrhagic serotype O157:H7), surface antigens (endotoxin, flagellar protein) for serotype O157:H7, and Shiga-like toxins (Stx1 and Stx2) utilized as bio-recognition moieties, which are comprehensively explored one-by-one during the doctoral studies. The objective was also to explore methodologies for aptamer generation and the simultaneous synthesis of nanostructures relevant for aptasensing via spectroscopic and electrochemical tools. Thus, in line with our aim, various fully characterized DNA aptamers with an excellent affinity (nanomolar K d) to the selected bacterial targets were successfully screened ifrom a naïve oligomer library of 81 nucleotides with 45 nucleotide random region, implementing indigenous in-vitro selection SELEX (systematic evolution of ligands by exponential enrichment) methodologies of oligomer panning for the selected targets: (i) Microtiter cell-SELEX using phenylboronic acid as a capturing agent for E. coli bacteria accompanied by counter screening to mitigate aptamer cross-talk with other closely related bacterial species (K d of 14 nM for O78:H11 and 69 nM for O157:H7 serotype, respectively) was developed. (ii) Competitive microtiter-SELEX employing rival ligands for raising stringency for purified E. coli O157:H7 endotoxin and flagellar proteins (K d of 5.3 and 4.6 nM, respectively) was developed. (iii) Biolayer interferometry (BLI)-SELEX using Octet Red96 system, a one-step technique for rapidly generating aptamers against E. coli Shiga toxin subtypes viz., Stx1 & Stx2 via specific epitopic peptides (K d of 47 pM & 29 pM, respectively) in a dip microtiter plate format, obliterating the need for multiple oligomer enrichment cycles was designed and demonstrated. Parallelly, novel nanomaterials of various nano-dimensions (0D, 1D, 2D, 3D) exhibiting excellent transducer properties were synthesized and characterized using microscopic (scanning electron microscopy, transmission electron microscopy), spectroscopic (ultraviolet-visible and fluorescence spectroscopy, confocal Raman spectroscopy, Fourier transform Infra-red spectroscopy, X-ray diffraction and, energy-dispersive X-ray elemental mapping), electrochemical (cyclic voltammetry, impedance spectroscopy, square wave voltammetry) and other analytical techniques (dynamic light scattering, zeta potential and Brunauer–Emmett–Teller (BET) surface area analysis) techniques. The specific aptamers conjugated with enhanced nanostructures conferred both selectivity and sensitivity in spiked water samples and complex matrices like juices and sera, with minor pre-processing steps mentioned in respective chapters. The following biosensing platforms were demonstrated for the selected targets: (i) Label-free impedimetric sensing of E. coli O78:H11 was successfully demonstrated using novel selected aptamer functionalized bridged rebar graphene (synthesized by un-scrolling of multiwall carbon nanotubes and bridged using terephthalaldehyde forming 3D nano-construct) onto disposable screen-printed electrodes demonstrating a limit of detection ~ 10 cells and a dynamic response range from 10 1 to 10 6 cells. (ii) Label-free impedimetric sensing of E. coli O157:H7 was showed using specific aptamer functionalized boron-carbon nanorods decorated by nickel nanoparticles with a similar limit of detection and a dynamic detection range of 10 0 to 10 5 cells in water and juice samples. (iii) Fluorescence 'turn on' bioassay based on fluorescence quenching of aptamer functionalized carbon- dots by silver nanoparticles, which in the presence of the E. coli O157:H7 purified O-antigen and H-antigen was recovered, showing a limit of 0.12 pg mL -1 , a wide dynamic range of detection (1 pg mL -1 – 10 ng mL -1) and the stable response recorded even in pure water. (iv) A voltammetric diagnostic assay via aptamer functionalized onto chitosan exfoliated 2D tungsten diselenide (WSe 2) nanosheet platform showing a dynamic response range from 50 pg mL -1 – 100 ng mL -1 , and iidetection limit of 44.5 pg mL -1 & 41.3 pg mL -1 for Stx1 and Stx2, respectively, which showed minimal cross- reactivity in spiked sera samples. These nanostructured aptasensors showed quick results, negating the enrichment of bacterial load in test samples as required in conventional systems. The aptamers also showed admirable application as capture & detection for bacterial populations onto soft-lithographed polydimethylsiloxane based microfluidic biosensing platforms. In toto, this thesis addresses the issues of conventional detection of enteric pathogenic E. coli species and, the unique detection methodologies presented in the studies can be further extended to other clinically or environmentally relevant bacterial species, antigens or biomolecules, including viral diseases like COVID-19, hepatitis, and influenza. The scientific works included herein are advantageous for the development of analytical platforms using cost & reagent-effective protocols for pathogen or bio-analyte determination and thus, holds promising future perspectives in the field of in-vitro clinical diagnostics.

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