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An electrochemical immunosensor for efficient detection of uropathogenic *E. coli* based on thionine dye immobilized chitosan/functionalized-MWCNT modified electrode

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ABSTRACT

Uropathogenic *Escherichia coli* (UPEC) is the major cause of 150 million Urinary Tract Infections (UTI) reported annually world-wide. High prevalence of multi-drug-resistance makes it dangerous and difficult to cure. Therefore simple, quick and early diagnostic tools are essential for effective treatment and control. We report an electrochemical immunosensor based on thionine dye (Th) immobilized on functionalized-multiwalled carbon nanotube+chitosan composite coated on glassy carbon electrode (GCE/f-MWCNT-Chit@Th) for quick and sensitive detection of UPEC in aqueous solution. This immunosensor was constructed by sequential immobilization of UPEC, bovine serum albumin, primary antibody and Horse Radish Peroxidase (HRP) tagged secondary antibody on the surface of GCE/f-MWCNT-Chit@Th. When analyzed using 2.5 mM of hydrogen peroxide reduction reaction using cyclic voltammetry in phosphate buffer, pH 7.0, the immunosensor showed excellent linearity in a range of $10^2 - 10^9$ cfu of UPEC mL^{-1} with a current sensitivity of 7.162 μA $\{\log(\text{cfu mL}^{-1})\}^{-1}$. The specificity of this immunosensor was tested using other UTI and non-UTI bacteria, *Staphylococcus*, *Klebsiella*, *Proteus* and *Shigella*. The clinical applicability of the immunosensor was also successfully tested directly in UPEC spiked urine samples (simulated sample).

1. Introduction

Early and affordable rapid detection of pathogenic bacteria is an important healthcare requirement and unfulfilled millennium goal (Pruss-Ustun et al., 2008). This demand is only growing rapidly, as apparently harmless bacteria like *Escherichia coli* (*E. coli*) are turning into multi-drug-resistant pathogens and getting widely distributed in humans, other warm-blooded animals and the environment. Cholecystitis, bacteremia, cholangitis, urinary tract infection are common *E. coli* infections (Edberg et al., 2000). Among these, yearly 150 million people are seriously affected by urinary tract infections (UTI), mostly women and diabetic patients. Half of the incidence is caused by Uropathogenic *E. coli* (UPEC). Conventional methods involve selective culturing and isolation of cells followed by biochemical and serological methods for detection and identification. Although these methods are reliably accurate, time-consuming multiple-step procedures (2–3 days) and requirement of skilled personnel are major disadvantages (Edberg et al., 2000; Clesceri et al., 1998; Ivnitski et al., 1999). Modern techniques like polymerase chain reaction (PCR) (Hoshino et al., 2004; Lazaro et al., 2003; Mohammadi et al., 2005; Greisen et al., 1994), flow-cytometry (Hibi et al., 2006; Kempf et al., 2005) and mass spectrometry (Tseng et al., 2012) do provide technology solution for early and sensitive detection. However, apart from prohibitively expensive instrumentation, these require experienced technicians and costly reagents and hence not widely used in clinical practice. Biosensors with affordable instrumentation appear to be the best alternative at the moment.

Conventionally microbial biosensors are used to measure dissolved oxygen, glucose, alcohol, hydrocarbon etc. in the medium to deduce the microbial load (D'Souza, 2001). Recently immunosensors (based on specific antigen-antibody interaction) have been developed for selective detection of a bacterium

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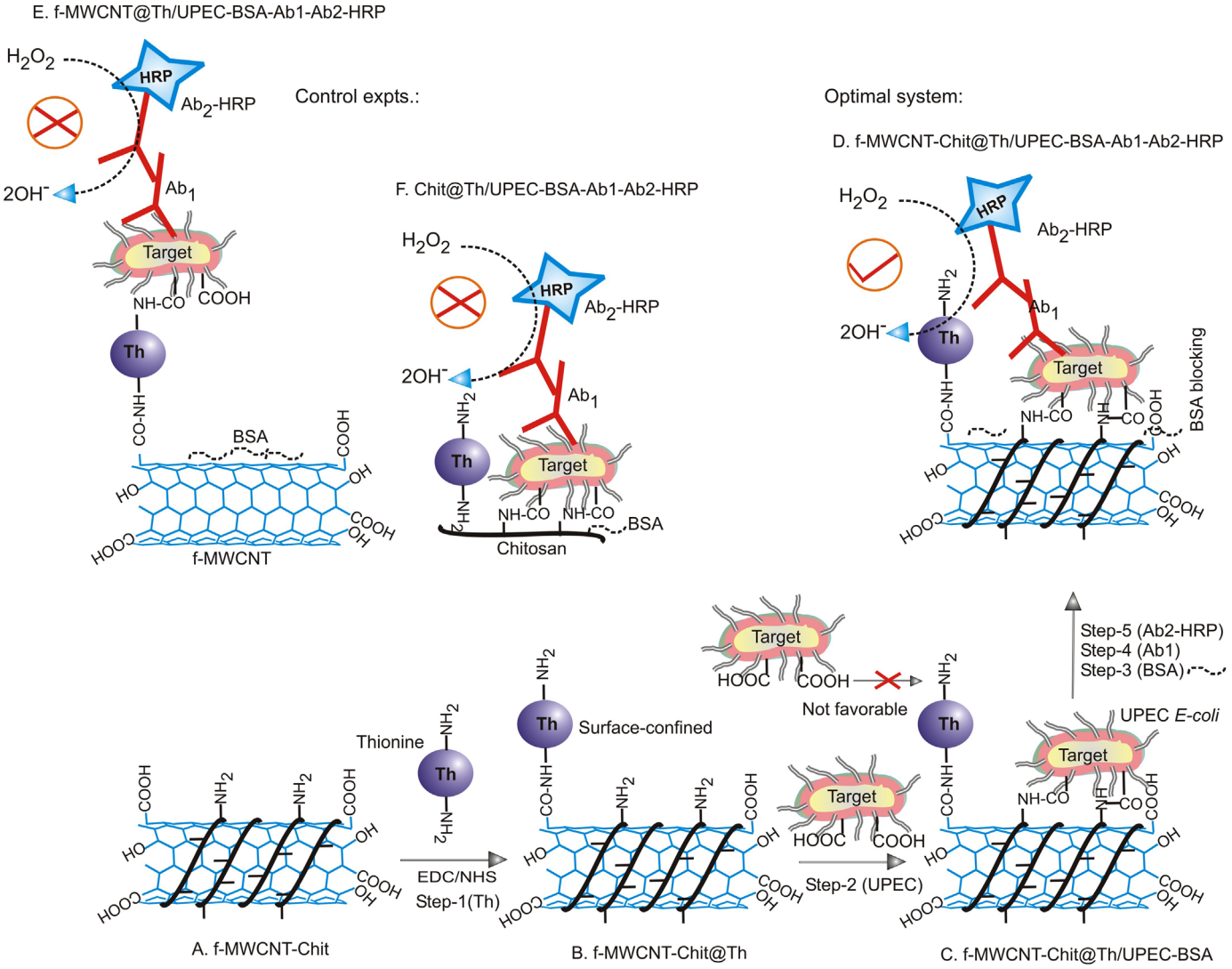
(Ivnitski et al., 1999). Transducing techniques such as surface plasma resonance (Si et al., 2011), quartz crystal microbalance (Temur et al., 2010), piezoelectricity (Guo et al., 2012), bioluminescence (Prosser et al., 1996; Ramanathan et al., 1998), optical absorbance (Ivnitski et al., 1999), fluorescence (Jiang et al., 2008) and electrochemistry (Kavosi et al., 2014) have been utilized in accompanying instrumentation development. Among these, electrochemical immunosensors are attractive owing to affordable instrumentation, ease-of-use, cost effectiveness and portability (Li et al., 2008; Preechaworapun et al., 2008). Generally, immunosensors make use of labelled- and label-free detection strategy. Antibodies labelled with the enzyme horseradish peroxidase (HRP) capable of generating electro-active species for sensitive analysis is quite common and often used in laboratories and clinical practice (Su et al., 2010; Wang et al., 2011; Yu et al., 2006). Label-free immunosensors require many operational steps, expensive reagents and their sensitivity is lower (Wang et al., 2013). Though there are many reports on electrochemical detection of various *E. coli* strains, there is none reported for the whole-cell UPEC detection, possibly because of requirement of selective pre-enrichment and interference from several electro-active species such as uric acid, cysteine, xanthine, hypoxanthine and urea in urine sample. We report a simple immunosensor developed using indirect ELISA technique for rapid detection of UPEC in urine sample analysis.

Among nanomaterials such as carbon nanoparticles (Du et al., 2010) especially carbon nanotubes (CNT) (Yu et al., 2006; Akter et al., 2012), gold nanoparticles (Jeong et al., 2013), silica nanoparticles (Lin et al., 2011) and magnetic nanoparticles (Mani et al., 2009; Li and Gao, 2008), CNT has acquired great attention due to its physical properties, chemical stability and remarkable conductivity (Katz and Willner, 2004). Immobilization of antibody molecules without leaching from the electrode surface is crucial to development of efficient immunosensor system. Oxygen-functionalized multiwalled carbon nanotube (f-MWCNT, f=functionalized) is found to provide an excellent platform for stable immobilization via covalent linkage between the oxygen functional groups of f-MWCNT and amino groups of the antibody (Pauliukaite et al., 2009). Since non-homogeneity of the MWCNT surface is a major concern, MWCNT dispersed in polymers like Chitosan, Nafion and ionic liquids provide near homogeneity (Huang et al., 2010; Chen et al., 2009; Buzzeo et al., 2004). There have been few reports relating to MWCNT, gold nanoparticle and thionine dye based immunochemical biosensor (detection of Pseudomonas aeruginosa, Listeria monocytogenes and allergic food proteins), in which a dilute solution of thionine (Th) dye was taken as a solution phase



Scheme 1. Illustration for the preparation of UPEC electrochemical immunosensor based on f-MWCNT-Chit@Thionine chemically modified electrode (Step-1) by sequential immobilization of UPEC cells (Step-2), Bovine serum albumin (Step-3), Ab_1 (Step-4) and Ab_2-HRP (Step-5) (A-D) and its relevant control experiments (E and F).