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Controlled deposition of functionalized silica coated zinc oxide nano-assemblies at the air/water interface for blood cancer detection

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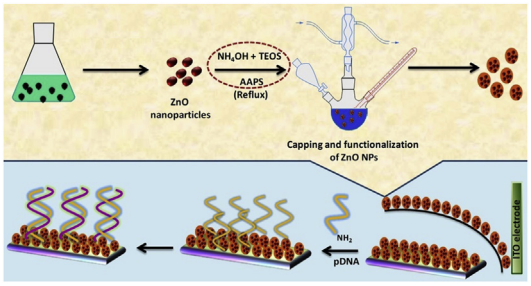
HIGHLIGHTS

- Stable and controlled deposition of Am-Si@ZnO nano-assemblies using LB technique.
- Uniform monolayer deposition of the Am-Si@ZnO LB film within the nanometer range.
- Am-Si@ZnO LB film shows enhanced electrochemical properties.
- Fabricated nucleic acid sensor is ultrasensitive (1×10^{-16} M) for CML detection.
- Validation with clinical samples of CML positive patients shows its potential for clinical diagnosis.

GRAPHICAL ABSTRACT



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ABSTRACT

We report results of the studies relating to controlled deposition of the amino-functionalized silica-coated zinc oxide (Am-Si@ZnO) nano-assemblies onto an indium tin oxide (ITO) coated glass substrate using Langmuir-Blodgett (LB) technique. The monolayers have been deposited by transferring the spread solution of Am-Si@ZnO stearic acid prepared in chloroform at the air-water interface, at optimized pressure (16 mN/m), concentration (10 mg/ml) and temperature (23 °C). The high-resolution transmission electron microscopic studies of the Am-Si@ZnO nanocomposite reveal that the nanoparticles have a microscopic structure comprising of hexagonal assemblies of ZnO with typical dimensions of 30 nm. The surface morphology of the LB multilayer observed by scanning electron microscopy shows uniform surface of the Am-Si@ZnO film in the nanometer range (<80 nm). These electrodes have been utilized for chronic myelogenous leukemia (CML) detection by covalently immobilizing the amino-terminated oligonucleotide probe sequence *via* glutaraldehyde as a crosslinker. The response studies of these fabricated electrodes carried out using electrochemical impedance spectroscopy show that this Am-Si@ZnO LB film based nucleic acid sensor exhibits a linear response to complementary DNA (10^{-6}

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-10^{-16} M) with a detection limit of 1×10^{-16} M. This fabricated platform is validated with clinical samples of CML positive patients and the results demonstrate its immense potential for clinical diagnosis.

1. Introduction

Leukemia is a progressive disease wherein the bone marrow and other blood-forming organs produce increased numbers of abnormal leukocytes, leading to anemia and other manifestations [1]. Chronic myeloid leukemia (CML) is one of the most common type of leukemia, which is induced by the active breakpoint cluster region (BCR)/Abelson (ABL) oncogene [2]. The abnormality is produced by reciprocal translocation of chromosome 9 and 22 and it usually occurs in more than 95% of the CML patients [3,4]. The molecular analysis of this translocation shows that the breakpoints on chromosome 22 occur within 5.8 kilobases (kb) of the breakpoint cluster region (BCR) gene [3]. Thus, BCR/ABL is an important biomarker for early diagnosis of the disease, and for detection of minimal residual leukemia cells in the CML patients, especially after the bone marrow transplantation [5]. The conventional techniques like chromosome analysis, fluorescence *in situ* hybridization, flow cytometry, real-time quantitative reverse transcription polymerase chain reaction can be used for the BCR/ABL fusion gene detection [6]. However, some of the major drawbacks of these techniques include poor precision, cost and long delay etc. [7].

The electrochemical DNA biosensors based on nucleic acid hybridization have recently been explored for diagnosis of blood cancer [8–10]. DNA biosensors offer a new approach for rapid, sensitive, simple and low-cost detection of specific nucleic acid sequences [9,11]. Some electrochemical DNA sensors based on quantum dots (QD) and conducting polymers have been reported for detection of BCR/ABL oncogene [12,13]. It has been found that the immobilizing matrix plays an important role towards the fabrication of a biosensor [14,15]. In this context, an important issue for QD application in biosystems is the short/long-term toxicity of QDs [16]. Quantum dots are known to damage DNA and reduce the activity due to factors such as the surface coating [17]. Lin et al. fabricated an electro-chemical DNA biosensor based on glassy carbon electrode using methylene blue (MB) as the hybridization indicator [18]. This sensor shows the detection limit of 5.9×10^{-8} M. An impedance biosensor for detection of CML, based on DNA immobilized gold nanoparticles/gold modified electrode with a detection limit of 1.0×10^{-12} mol L^{-1} was fabricated by Ensafi et al. [19]. However, validation with the clinical patient samples was not reported. In spite of these developments, there is an urgent need for the availability of a sensitive, specific and mediator free reusable biosensor that can be used for early and reliable detection of CML.

The assembly of a modified material onto an electrode surface is crucial for the construction of an electrochemical biosensor as biomolecules are known to get denatured quickly due to physiological changes [20–22]. For the fabrication of a high-performance miniaturized biosensor, an important step is the deposition of ultra-thin ordered structures because the performance of the device depends strongly on the molecular arrangement [23,24]. In this regard, the composition of a monolayer based matrix has been found largely based on the Langmuir–Blodgett (LB) technique that involves the transfer of a monolayer, assembled at the gas–liquid interface to a solid substrate [25,26]. Recently, nanostructured oxides of metals like zinc, iron, titanium, have been found to play a significant role since they provide desired orientation to the biomolecules with negligible conformational changes [27–29]. Among the various metal oxides, zinc oxide nanoparticles (ZnONPs) due to their easy fabrication, biocompatibility, environmental friendly nature, and non-toxic synthesis route can be used for biosensor application [30–32]. Zinc oxide is a wide band gap semiconductor (Eg = 3.37 eV), which makes it more attractive for fabrication of high-efficiency nano-devices because of its high exciton binding energy of 60 meV and high mechanical and thermal stabilities [33,34].

We report results of studies relating to the fabrication of a novel electrochemical DNA sensor based on amino-functionalized silica coated ZnONPs (Am-Si@ZnO NPs) using LB technique. This platform has been utilized for the detection of BCR-ABL fusion gene of CML by covalently immobilizing probe sequence specific to CML. This optimized Am-Si@ZnO nano-assembly shows excellent specificity for single-base mismatch and complementary DNA after hybridization and the specific probe has been used for the assay of a PCR clinical sample of CML patient.

2. Material and methods

2.1. Chemicals

Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), tetraethyl orthosilicate (TEOS, 98%), n-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AAPS, 80%), lithium hydroxide ($LiOH \cdot H_2O$), glutaraldehyde were purchased from Sigma–Aldrich (USA). The other reagents were of analytical grade and were used as received. CML specific probe oligonucleotide sequence, complementary, non-complementary and one-base mismatch target sequence used in this work are procured from Sigma-Aldrich, USA and are as follows.

Probe DNA (pDNA): Amine–5′–TGT CCA CAG CAT TCC GCT GACC–3′
Complementary: 5′–GGT CAG CGG AAT GCT GTG GACA–3′
One-base mismatch: 5′–GCT CAG CGG AAT GCT GTG GACA–3′
Non-complementary: 5′–TAC TCG CAA TAA CGT GAT CTCC–3′

The reagents were prepared in deionized water (Millipore, 18.0 MΩ cm), and the solutions and glassware were autoclaved prior to use. Tris-E buffer (1 M Tris-HCl and 0.5 M EDTA, pH 8.0) was used to prepare all oligonucleotide solutions and stored at −20 °C.

2.2. Synthesis of zinc oxide nanoparticles

Zinc oxide nanoparticles (ZnONPs) were synthesized by a previously reported method with slight modifications [35,36]. The alkaline solution was prepared by dissolving $Zn(NO_3)_2 \cdot 6H_2O$ and LiOH of equal concentration (0.1 M) in water and ethylene glycol (5:1) [37]. The solution was kept for 6 h without stirring, and the temperature was maintained at 100 °C. After the hydrolysis reaction, the white precipitates thus formed were collected by centrifugation and thoroughly washed with deionized water (5 times) and dried in vacuum oven (60 °C) [35].