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Electrochemical aptasensor design for label free cytosensing of human non-small cell lung cancer

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ABSTRACT

A roboust aptamer based electrochemical cytosensing platform for detection of adenocarcinoma type lung cancer based on electrochemical impedance spectrometry (EIS) was developed with high sensitivity, selectivity and devoted to medical applications. Screen printed carbon electrodes (SCPEs) were used as sensor surface, 5'amino linked aptamer sequence was immobilized onto the SPCEs, interaction with cancer cells at 37.5 °C was monitored by EIS transduction of the Rct in the presence of 5 mM $[Fe(CN)_6]^{3-/4-}$. Designed aptasensor's selectivity was determined by using Human liver hepatocellular carcinoma (HepG2) and human cervical cancer (HeLA) cells. The purposed cytosensor showed high sensitivity with a detection limit of 163.7 cells/mL. This simple, rapid and low cost electrochemical approach offered a label free detection with capable of early detection in cancer diagnosis with interest for future applications.

1. Introduction

Cancer is still one of the major public health problem worldwide. It is the second leading reason of death following cardiovascular diseases [1]. Studies focusing on cancer prevention, early detection, and treatment aim to decline the cancer death rate.

Lung cancer is one of the leading cause of cancer-related mortality [2]. In USA lung cancer accounts for 29% of all cancer deaths, and it has a 5-year survival rate less than 15% [3]. Therefore, early detection is especially important for survival of patients. There are two histological subtypes of lung cancer which are non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). SCLC has highest tendency for early dissemination with low survival rate over five years [4–5]. On the other hand 80% of lung cancer patients are diagnosed with NSCLC [6].

Early detection of lung cancer is critical since 5-year survival rate decrease from 60% to 1% at clinical stage I and IV, respectively [7]. Today several imaging techniques such as spiral computed tomography (CT), positron emission tomography (PET), autofluorescence bronchoscopy are used for detection of lung cancer. However these approaches are not sufficient enough for detection of premalignant stage of lung cancer probably due to limitation and low sensitivity of morphological criteria used in these techniques [8–9].

Aptasensors hold great promise in biosensing techniques devoted to medical applications for last decade [10]. These synthetic nucleic acid sequences act as antibodies in binding biological targets such as, proteins [11–13], enzymes [14], toxins [15], and cells [16–17] due to their high affinity and high stability.

Electrochemical aptasensors provide a unique platform for clinical analysis with the advantages of high selectivity, high sensitivity and low cost detections [18–19].

Several electrochemical methodologies have been developed for cancer cell cytosensing. Lv et al. used He-3 antibody molecules for detection of MCF-7 cell lines [20], Zhang et al. developed leukemia sensitive aptamer based electrochemical cytosensor by using magnetic nanoparticles, in a detection limit of 10 cells [21].

In addition Electrochemical Impedance Spectormetry (EIS) based aptasensors also offer label free detection additionally [22]. Electrochemical impedance spectroscopy (EIS) is based on interfacial investigations thus is a unique analytical device for label free diagnostic analysis. EIS measures the response of an electrochemical system to an applied oscillating potential as a function of the frequency. Impedimetric techniques have been developed to characterize the fabrication of the sensors and to monitor the biochemical reaction occures at the sensor surface [23–24].

Non- small cell adonocarcinoma type lung cancer detection by using a label free impedimetric cytosensor assay at aptamer modified screen printed carbon electrode surfaces (SCPEs) was performed first time in this study. Human hepatic carcinoma and human cervical carcinoma type cell cultures were also used as negative control for the detection of aptasensor selectivity. The results obtained from the study shows that the designed aptasensor is sensitive and selective enough, capable

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of performing cost- effective, fast, reliable and label free detection. In addition by using this methodology different cancer types could be detected in clinical applications.

2. Materials and methods

2.1. Chemicals and reagents

N-hydroxysulphosuccinimide (NHS), [*n*-(3-dimethylamino)propyl)]–*N*-ethylcarbodiimide (EDC), ethanolamine were purchased from Sigma-Aldrich Chemical Company (Germany).

DNA aptamer that selective to A549 cell culture (human lung cancer) 5′ amino modified was purchased from TIB Molbiol (Germany), The aptamer sequence has a following base composition [9].

5′NH_2 $(CH_2)_6$ GGT GCA TGC CGT GGG GAG GGG GGT GGG TTT TAT AGC GTA CTC AG 3′.

Three cancer cell lines, A549 (human non-small cell lung cancer), HeLA (human cervical cancer) and HepG2 (human hepatocellular cancer) are from American Type Culture Collection (ATCC) (Rockville, MD, USA).

Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), L-Glutamine, Trypsin-EDTA solution and Trypan Blue were purchased from Biological Industries (Israel). Metallized Hemacytometer Reichert Bright-Line Counting chamber was purchased from Hausser Scientific.

2.2. Apparatus

Electrochemical Impedance Spectrometric (EIS) measurements were performed by using AUTOLAB PGSTAT-30 electrochemical analysis system (Eco Chemie –The Netherlands). Screen printed carbon electrode containing the three electrode system as working electrode, an Ag/AgCl reference electrode, and a auxiliary electrode were purchased from Dropsens (Spain).

Culturing of cells were performed using laminar flow hood (ESCO, USA) and water jacketted CO2 incubator (Thermo, USA). Counting of cells and morphological analysis are performed using an inverted microsope Olympus CKX41 (USA).

2.3. Methods

2.3.1. Cell culture

All cells were cultured and maintained according to ATCC's instruction in DMEM medium supplemented with %10 FBS and 2 mM L-Glutamine. All cells were cultured as monolayers and maintained at 37.5 °C in humidified 5% CO2 incubator. All cells were cultured in 100 mm cell culture dishes. For electrochemical sample preparation the cell layer is rinsed first with 0.05% Tripsin-0.53 mM EDTA solution to remove serum traces. Then cells were treated with 2 mL of Tripsin-EDTA solution until cells were dispersed. After addition of 7 mL complete DMEM medium cells are aspirated by gently pipetting. Cells were centrifuged at 200 x g for 2 min and cell pellet was suspended with 1 mL complete DMEM medium. Cells were counted by using Trypan Blue exclusion staining under inverted microscope using hemacytometer. Total number of cells per milliliter was calculated and diluted with cell medium as desired.

Fig. 1 shows the morphology of the used cell lines obtained from ATCC.

2.3.2. Aptasensor preparation

2.3.2.1. Electrode surface modification. SCPE surfaces were chemically modified by exposure to 0.5 M phosphate buffer containing 5 mmoL/L EDC and 8 mmoL/L NHS used for free amino group coupling.

2.3.2.2. Aptamer biomodification. 5′ amino terminated A549 cell sensitive aptamer was immobilized on to modified SCPE surfaces by immersing the modified electrodes in to desired concentration of the aptamer solution, in acetate buffer pH 4.8, for 1 h. After that, the modified electrodes were immersed in %1 diethanolamine solution during 1 h to prevent non specific adsorptions.

2.3.2.3. Aptamer cell interaction. Aptamer biomodified electrodes were immersed into PBS solution including 0.5 cell/mL $\times 10^5$ of A549 lung cancer cell cullture at 37.5 °C by shaking for 1 h and washed with PBS for 1 min to remove unbound cells. Aptasensor selectivity was performed by using human hepatocarcinoma (HepG2) and human cervical cancer (HeLA) cell cultures as target by following the same protocol.

2.3.2.4. Impiedimetric transduction. EIS used for thrombin detection. Modified SPE's are measured in a PBS (pH 7.4) in the precense of 0.5 mM Fe[CN6]3 −/4− at +0.24 V. A frequency range from 10 kHz to 50 mHz and an AC amplitude of 10 mV were applied. The impedance data was fitted to an equivalent circuit.

All results reported in this paper are the means of at least five measurements and the error bars are the corresponding standard deviations.

3. Results and discussion

A label free impedimetric aptasensor device has been developed for sensitive detection of non- small cell lung cancer based on aptamer – cell interaction. 5′ amino hexyl linked aptamer sequence was immobilized onto SCPE surfaces via carbodiimide chemistry, after surface blockage with ethanolamine, aptamer- cell interaction was monitorized by using EIS. A general scheme of the experimental procedure is shown in Fig. 2.

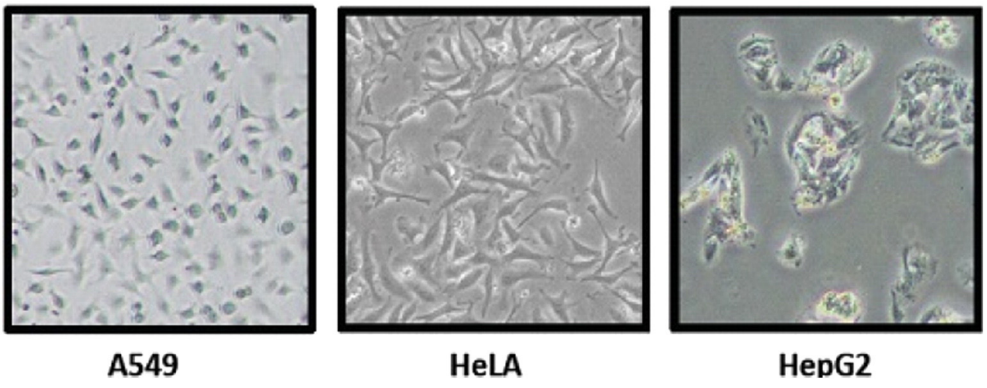

Fig. 1. Morphology image of A549, HeLA and HepG2 cells.