Biosensors and Bioelectronics 86 (2016) 33–40

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Enhanced electrochemical sensing of leukemia cells using drug/lipid co-immobilized on the conducting polymer layer

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ARTICLE INFO

Article history:
Received 28 April 2016
Received in revised form
9 June 2016
Accepted 10 June 2016
Available online 11 June 2016

Keywords:
Leukemia cells
Cancer
Anticancer drug molecules
Folate receptors
Conducting polymer

ABSTRACT

Electrochemical biosensors using five anticancer drug and lipid molecules attached on the conducting polymer layer to obtain the orientation of drug molecules toward cancer cells, were evaluated as sensing materials and their performances were compared. Conjugation of the drug molecules with a lipid, phosphatidylcholine (PC) has enhanced the sensitivity towards leukemia cells and differentiates cancer cells from normal cells. The composition of each layer of sensor probe was confirmed by electrochemical and surface characterization experiments. Both impedance spectroscopy and voltammetry show the enhanced interaction of leukemia cells using the drug/lipid modified sensor probe. As the number of leukemia cells increased, the charge transfer resistance (R_{ct}) in impedance spectra increased and the amine oxidation peak current of drug molecules in voltammograms decreased at around 0.7–1.0 V. Of test drug molecules, raltitrexed (Rtx) showed the best performance for the cancer cells detection. Cancer and normal cell lines from different origins were examined to evaluate the degree of expression of folate receptors (FR) on cells surface, where cervical HeLa cell line was found to be shown the highest expression of the receptor. Impedance and chronoamperometric experiments for leukemia cell line (Jurkat E6-1) showed linear dynamic ranges of 1.0×10^3–2.5×10^5 cells/mL and 1.0×10^3–8.0×10^3 cells/mL with detection limits of 68 ± 5 cells/mL and 21 ± 3 cells/mL, respectively.

1. Introduction

Cancer is one of the deadliest abnormalities in the human evolution, which occurs due to genetic and epigenetic alterations of the cells, hence it is of paramount importance to detect the cancer at an early stage. Diagnosis of cancer is practically made by the microscopic observation of the cell or tissue sample by an expert. Lab tests of DNA, RNA and the cells' proteins can complimentarily help to know the presence of cancer (Bremnes et al., 2005). These methods involve tissue biopsy that is invasive and it takes long time to obtain the results. To complement the tissue biopsy, recent advancements in biomedical imaging, such as X-ray, MRI, and CT scans gave powerful techniques, which provide a means for more efficient diagnosis of cancer. The major advantage of these techniques is accuracy; they all, however, suffer from the same disadvantages, such as high cost, long time to give results, and requirement of skilled labors. To overcome the disadvantages, a few methods for the detection of cancer cells, such as fluorescence *in-situ* hybridization (FISH) (Kumar and Pawaiya, 2010), comparative genomic hybridization (Shi et al., 2013), and electrochemical techniques have been developed. Of these, electrochemical techniques provide a unique characteristic of being exceptionally sensitive, considerably fast, and highly cost effective. To date, various electrochemical methods have been developed for the detection of cancer using the expression of the surface proteins as biomarkers (Chandra et al., 2013; Zhu et al., 2012; Lee et al., 2015; Han et al., 2014; Wu et al., 2015).

Most of the receptors such as folate receptor α are overexpressed on the cancer cells. Folate receptor α is overexpressed on the surface of various cancer cells and it can be used as a biomarker for cancer (Assaraf et al., 2014). Folate or vitamin B9 is a biologically inactive compound, but when converted to tetrahydrofolate in the liver, it becomes biologically active. Folate is needed for the biosynthesis of both purines and pyrimidines, which are the basic units of both DNA and RNA. In addition, it is required for the methylation of DNA as well as to act as a cofactor in certain biological reactions, such as one-carbon metabolism pathways (Weinstein et al., 2003). Since cancer cells are fast dividing cells, they need the active intake of folate, which is

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http://dx.doi.org/10.1016/j.bios.2016.06.029
0956-5663/

achieved by the overexpression of FR on the cell surface (O'Shannessy et al., 2011). However, cells of myeloid leukemia, which are folate positive, show a steady expression of FR on the cell membrane and are undetectable using conventional methods such as immunohistochemistry (Simmons et al., 2003; Franklin et al., 1994; Lynn et al., 2015; Levy et al., 2003). In order to overcome this, we have devised an electrochemical, drug-lipid conjugate based on the conducting polymer that shows enhanced sensitivity towards folate positive leukemia cancer cells. Furthermore, the expression of FR-β on the surface of leukemia cells was increased by the external stimulation with *all-trans* retinoic acid (ATRA) to study the specificity and concentration dependency of the sensor (Wang et al., 2000). The present work surpasses the previously reported works targeting folate receptors, by being high sensitive and capable of direct electrochemical detection (Castillo et al., 2013; Zhao et al., 2013).

One of the major difficulties in the direct voltammetric analysis of folate or any of the amine containing molecules is the fouling of the electrode surface by the irreversible reaction of amine to imine or to produce other by-products. Hence, many different materials and methods have been employed by the researchers around the world to nullify the electrode surface silencing (Manica et al., 2003). In the present study, we tried to minimize the fouling of the electrode surface during the direct measurement by modifying the electrode surface employing the organic π-conjugated conducting polymers (Rahman et al., 2003, 2005) and the conjugation of lipid molecules (Kwon et al., 2006). Direct electrochemical detection of folate positive cancer cells is one of the significant criterions for the future development of real time cancer detection methods and routine screening of cancer using the FR as a biomarker.

The present study deals with the enhanced electrochemical detection of leukemia cells by using lipid and anticancer drug conjugated on a conducting polymer layer. The signal enhancement was obtained by increasing the expression of the receptors on the cell surface by external stimulator. The study also includes comparative work between five anticancer drug molecules with lipids for their interaction with the cancer cells. The amine functionalized organic conducting polymer [2,2′:5′,2″-terthiophene]-3′,4′-diamine (DATT), was used as an electrode material and as a substrate for the drug and lipid immobilization. Furthermore, the sensor was characterized by CV, impedance, and XPS analysis and optimized in terms of different analytical parameters affecting the sensor performance such as pH, temperature, concentration of drug molecules, and the formation of the polymer film on the electrode surface. The sensor was demonstrated to quantify the level of expression of receptors on the surface of different cell lines and to successfully detect cancer cells.

2. Experimental

2.1. Materials and apparatus

All the chemicals used in the study were of extra pure quality obtained from Sigma Aldrich and used as received. All the electrochemical experiments were conducted in a three electrode system using a Kosentech PT-1 potentiostat/galvanostat (S. Korea). The surface characterization of the sensor probe layer was done using XPS and FE-SEM at Korea Basic Science Institute (KBSI). The other instrumental experiments were described in detail in the supplementary material (S1, S2, and S3).

2.2. Cell culture and detection procedures

The cancerous HeLa, A549, and MDA-MB-231 cells were cultured in a T75 culture flask in Dulbecco's modified eagles medium supplemented with 10% heat inactivated fetal bovine serum, 100 units/mL of penicillin and 100 units/mL of streptomycin at 37 °C under 5% CO_2 and 95% humidity in the incubator. The normal MCF-10A cells were cultured in a modified DMEM/F12 medium supplemented with 5% horse serum, 20 ng/mL of epidermal growth factor and 10 μg/mL of insulin along with 10% FBS and 100 units/mL of penicillin/streptomycin. Human acute T cell leukemia (Jurkat E6-1 clone) cells were cultured as suspension cultures in a RPMI-1640 medium supplemented with 10% FBS and 100 units/mL of penicillin/streptomycin. The media of cultures were replaced once in every two days until 95% confluency was obtained and then sub-cultured. For the interaction with the proposed electrochemical biosensor the cells were removed from culture conditions following trypsinization and suspended in 0.1 M PBS. The resulting cell suspension was washed with PBS three times to remove any remaining culture media. Before taking the voltammetric and impedometric measurements the GC/pDATT-Rtx+PC sensor probe was incubated for 25 min with the buffer solution containing cancer cells and the non-cancerous MCF-10A cells separately to study the extent of their interaction. After incubation with the cancer cells, the sensor was subjected to different experiments to detect cancer cells captured on the surface via FRs. Since the live cells are very sensitive towards the pH and temperature changes, the optimum temperature of 37 °C and the pH of 7.4 were maintained throughout the experiment so that the cells do not lose their integrity.

3. Results and discussion

The biosensor was constructed in a few modification steps, where the drug molecules were covalently immobilized on the amine functionalized polyterthiophene formed by electrochemical polymerization on the electrode surface, alternatively the drug molecules were co-immobilized along with the lipid molecule, which interacted with the drug molecules through hydrophobic interaction to form a homogeneous mixture and simultaneously attached on the amine groups of the polymer along with the drug molecules, for increased sensitivity as shown in Scheme 1. After the immobilization of drug molecules on the polymer modified surface, the CVs show irreversible anodic peaks at 0.66 V (raltitrexed), 0.68 V (pemetrexed), 0.79 V (folinic acid), 0.90 V (folic acid), and 0.97 V (methotrexate) corresponding to the oxidation of the amine groups present in different drug molecules (Fig. 1(A)). Among the five drug molecules, Rtx showed the highest anodic peak current with better stability at the lowest oxidation potential when compared to the others (Fig. 1(A)). Differently charged lipid molecules were tested to check their impact on the voltammetric response of the sensor surface, as shown in Fig. 1(A) the positively charged PC shows better CV response along with a decreased electrode fouling (Park et. al., 2002; Cheng et al., 2004). The sensor probe thus fabricated was characterized by voltammetry, impedance spectrometry, and XPS experiments. Cancer cells were identified by the interaction between Rtx on the electrode surfaces and the cell surface receptor.

3.1. Surface characterization of the sensor probe

To confirm the formation of each layer and to study the electrochemical behavior, CVs were recorded at each step of the sensor fabrication. During the electro-polymerization of the DATT monomer, two oxidation peaks were observed at 0.76 and 1.53 V (Fig. S1(A)). After polymerization, CVs were recorded in a blank PBS solution (pH-7.4) to observe and confirm the electrochemical behavior as shown in Fig. S1(B(a)). However, no interfering peak for amine group of monomer was observed. The FE-SEM image of