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Electrochemical approach for monitoring the effect of anti tubulin drugs on breast cancer cells based on silicon nanograss electrodes

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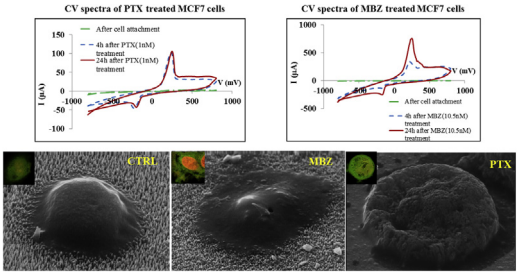
HIGHLIGHTS

- Electrochemical effect of MBZ and PTX (anti tubulin drugs) on breast cancer cells was detected.
- Detection was carried by silicon nanograss electrodes(SiNGEs).
- Signaling pathways activated in the cells by drug treatment, change the anodic/cathodic response of cells covered SiNGEs.
- Cytochrome C and ERK$^{1/2}$ play the crucial role in changed electrochemical responses of MBZ and PTX treated cells respectively.

GRAPHICAL ABSTRACT



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ABSTRACT

One of the most interested molecular research in the field of cancer detection is the mechanism of drug effect on cancer cells. Translating molecular evidence into electrochemical profiles would open new opportunities in cancer research. In this manner, applying nanostructures with anomalous physical and chemical properties as well as biocompatibility would be a suitable choice for the cell based electrochemical sensing. Silicon based nanostructure are the most interested nanomaterials used in electrochemical biosensors because of their compatibility with electronic fabrication process and well engineering in size and electrical properties. Here we apply silicon nanograss (SiNG) probing electrodes produced by reactive ion etching (RIE) on silicon wafer to electrochemically diagnose the effect of anticancer drugs on breast tumor cells. Paclitaxel (PTX) and mebendazole (MBZ) drugs have been used as polymerizing and depolymerizing agents of microtubules. PTX would perturb the anodic/cathodic responses of the cell-covered biosensor by binding phosphate groups to deformed proteins due to extracellular signal-regulated kinase (ERK$^{1/2}$) pathway. MBZ induces accumulation of Cytochrome C in cytoplasm. Reduction of the mentioned agents in cytosol would change the ionic state of the cells monitored by silicon nanograss working electrodes (SiNGWEs). By extending the contacts with cancer cells, SiNGWEs can detect minor signal transduction and bio recognition events, resulting in precise biosensing. Effects of MBZ and PTX drugs, (with the concentrations of 2 nM and 0.1 nM, respectively) on

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electrochemical activity of MCF-7 cells are successfully recorded which are corroborated by confocal and flow cytometry assays.

1. Introduction

Electrochemical biosensors are capable of transducing biochemical interactions to detectable electrical signals [1]. Biomarkers (e.g., enzymes, aptamers or antibodies), as the main part of the signal transmission, ought to be linked to the sensitive interface to mediate the sensing procedure [2,3]. However, limitations in types of suitable linkers, non-specific binding and complex chemical modifications perturb the reliability and accuracy of such biosensors. One of the newest challenges in electrochemical technology is replacing the marker based detection by label-free procedures. If the oxidative/reductive electrochemical responses of an analyte were unique in different biological transformations, we would achieve label-free sensing patterns.

Applying nanomaterials in interfacial modification of electrochemical biosensors greatly extended the range of response and enhanced the sensing performance depending on their sizes and shapes. Great physiochemical interactions with anomalous charge transfer ability were some of the characteristics reported for many nanostructures applied in electrochemical biosensing [3–6].

Moreover, if the biocompatibility of nanostructures would be acceptable, sensing interfaces produced by nanostructures would present new generation of label free biosensors for monitoring vital cells.

Among various biocompatible nanomaterials with good electron transport properties, silicon based nanostructures, such as silicon nanowire (SiNW), silicon nanotube (SiNT) and silicon nanograss (SiNG) were noticeably considered in biosensing approaches because of their greatly controllable conductivity [7,8], enlarged electrochemically active area and well compatibility with silicon fabrication processes [9]. Many electrical biosensors were developed based on SiNW arrays such as SiNW bio field effect transistor (bio FET) and SiNW based electrical cell impedance sensor (SiNW-ECIS) [10] for cancer cell detection as well as SiNW kinked transistor for signal extraction from cardiomyocyte cells [11]. Main disadvantage of SiNWs is using the gold catalyst in growth mechanism as a non-compatible material by FET fabrication process. Also engineering the size of SiNWs in low pressure chemical vapor deposition (LPCVD) or metal assisted etching systems wouldn't be simple. SiNGs are nanostructures which have been formed on silicon substrate with the assistance of reactive ion etching (RIE) as a simple and FET compatible process by fine aspect ratio and great charge transport properties [12]. It also presented well biological interaction with cells [13]. In this paper, we attempt to apply SiNG based electrochemical probes in interaction with breast cancer cells to assay the effect of anticancer drugs on their function and metabolism. The sensing has been based on monitoring the anodic/cathodic responses of the cell-covered probes after drug incubation. Formation of nanograsses on the surface of silicon substrate, increases the interactive surface with electrodes. Mebendazole (MBZ) and paclitaxel (PTX) are selected as anti-tubulin drugs to make non-regulated depolymerization and polymerization on cancer cells, respectively [14]. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) responses of breast cancer cells (MCF-7 cell line) are measured after their interaction with various doses of the drugs and the data is compared with control samples. Confocal and flow cytometry assays are also investigated as reference biological data to evaluate the validity of electrochemical results. SiNG probes serves as a good candidate for new generation of drug resistance biosensors in the field of cancer.

2. Materials and methods

2.1. Biosensor fabrication process

Silicon substrates were cleaned through standard cleaning method named RCA#1 (NH_4OH:H_2O_2:H_2O solution and volume ratio of 1:1:5 respectively). The surface of the wafer was then processed by RIE system (SensIran Co.) to form nanograss structures.

The grass formation was carried on by a radio frequency (RF)-plasma (13.56 MHz) in the ambient of mixed oxygen, hydrogen and sulfur-hexafluoride (SF6) gases with consecutive steps of passivation and etching sub-cycles [12]. H_2 and O_2 gases were mixed with the flows of 100 and 80 standard cubic centimeter per minute (SCCM) during the passivation step, respectively. A minor trace of SF_6 was also applied in this step. The plasma power and duration of the passivation step were 150 W and 50s, respectively. The etching step is conducted under the plasma power of 130 W for 10s using about 30 SCCM of SF_6 as inlet gas. Incorporating such procedure, various features are obtained on silicon substrates with sizes down to 100 nm in width. Finally, the nanograss substrate was located in phosphorous doping furnace to enhance the electrical conductivity of electrodes. The doping was conducted in the presence of Phosphorus oxychloride ($POCl_3$) in 850 °C.

2.2. Cell culture and drug incubation

MCF-7 cell lines obtained from the national cell bank of Iran (Pasteur Institute) had been isolated from grade I human breast tumors. Cells were maintained at CO_2 incubator (37 °C, 5% CO_2) in RPMI-1640 medium (Sigma) supplemented with 5% fetal bovine serum (Sigma) and 1% penicillin/streptomycin (Gibco). The fresh medium was replaced every other days. Prior to each experiment, cells were detached by trypsinization from the substrate and resuspended on the SiNG electrodes. To minimize the effect of trypsinization, the procedure was taken less than 4 min at room temperature around 20–22 °C. We held the samples in incubator for 4 h to achieve cells attachment on the SiNGs. Then, the MBZ and PTX drugs with various concentrations were added to individual samples. Such doses were 2.1 and 10.5 nM for MBZ and 0.1 and 1 nM for PTX drugs. The signal recording and biological assays were investigated 2, 6 and 10 h after addition of the drugs.

2.3. Electrochemical measurement procedure

Electrochemical CV test was performed using three electrode electrochemical cyclic voltammetry RNFPG Stat system (Roshd Nanofanavaran Co. Iran). SiNG electrode was utilized as working electrode (WE) in a fixed separation in the presence of standard reference electrode (RE) made by Ag/AgCl and counter electrode (CE) made by platinum. CV was performed between the SiNGWE and CE in the presence of RE biased at a fixed potential. Measurements were carried out at −0.8 to 0.8 V at a scan rate of 100 mV/s.