Impedance Monitoring of SH-SY5Y Cell Adhesion and Responses to As_2O_3 Treatment by Indium Tin Oxide Microelectrode Arrays

Hui Zhao, Fan Zhang, and Pingang He

***Abstract*— For most of the cells, cell adhesion is one of the essential factors for cell growth *in vitro*. In this paper, the impedance was used to monitor SH-SY5Y cell adhesion and evaluate the responses to As_2O_3 in a real-time, noninvasive, and label-free manner with indium tin oxide microelectrode arrays. Due to the transparency of the electrodes, cell activities can be observed simultaneously when impedance monitoring proceeded. The results indicated that the impedance responses had a good correlation with microscopic analysis of cell activities. Moreover, As_2O_3 at different concentrations were added for the evaluation of its effect on cell adhesion. 40 μmol/L As_2O_3 could detach almost all of the cells from the microelectrode surface, leading to an obvious decrease in impedance responses and declined cell survival rate after 9 h of treatment. Importantly, the impedance monitoring can provide the information about cell adhesion changes much earlier than commonly used cell microphotographs determination. This paper indicated the convenience and sensitivity of impedance for the direct and real-time monitoring of cell behaviors and drug-induced cell activities *in vitro*. It may be a driving force for the study of As_2O_3 effect on neuroblastoma and provides a useful and non-invasive analytical approach for cancer research.**

***Index Terms*— SH-SY5Y cell adhesion, As_2O_3 treatment, impedance monitoring, ITO microelectrode arrays.**

I. Introduction

FOR most of the cells, cell adhesion is integral to the survival and function displaying [1], [2]. It is the initial step of cell growth, migration, differentiation, metabolism and so on. If cell adhesion is delayed or damaged, the normal cellular properties and physiological functions cannot be maintained, and even induce the apoptosis of cells [3]. Therefore, the research of cell adhesion has great significance for understanding the biological and biomedical processes, such as wound healing, inflammation, vascular growth and metastasis of cancer [4]–[6].

Arsenic trioxide (As_2O_3) has been used as a medical agent for over 2000 years. It has been considered as a carcinogen for a long time, but in the later research, As_2O_3 had a good performance in cancer therapy through inducing cell apoptosis [7]. In the 1990s, in the treatment of refractory acute promyelocytic leukemia with As_2O_3, impressive clinical response rates was found and satisfying effect was achieved [8]. Since then, As_2O_3 has been widely applied to the treatment of numerous solid malignant tumors [9]–[11]. Neuroblastoma (NB) is a malignant childhood neoplasm of sympathetic nervous system, and previous studies have certificated that As_2O_3 can indeed inhibit the growth of NB cell lines and induce the apoptosis, which depend on the treatment time and dose [12]. SH-SY5Y cells derived from neoplastic tissues, is one kind of dopaminergic NB cell line [13]. They express several properties of mature sympathetic neurons and this cell line is good model to study neurodegenerative disorders [13]–[15]. However, there have been few studies concerning the effect of As_2O_3 on SH-SY5Y cell adhesion.

Traditionally, cell adhesion was analyzed by the microscopy-based methods [16]–[18], which often need fluorescent and chemiluminescent labeling, which would destroy the cells and lost very important biological information of cells. Therefore, there is a high demand for label-free detection methods. Previously, different techniques including resonant waveguide grating biosensors, optical waveguide lightmode spectroscopy, quartz crystal microbalance, impedance spectroscopy, etc., have been used for cell research [19]–[23]. Among them, cell impedance is a favored effective and low-cost technique, which can be used for continuous monitoring of cell activities. In this technique, cells are considered as dielectric particles, and cell adhesion and spreading on the working electrode can influence the current flow, leading to the increasing of impedance. In other words, the more active area of electrode surface is covered by cells, the larger impedance value will be detected [24]. The impedance changes are sensitive and can reveal relevant information about cell behaviors, such as adhesion, migration, differentiation, and cellular responses to chemical, biological, or other types of applied stimuli. Now, cell impedance has been developed and applied as one of the most interesting techniques to study cell adhesion, and supply real-time and kinetic information of cell biological processes [25]–[33]. During these processes, cell activities are always changing. If cell impedance monitoring and cell activities observation are achieved simultaneously and correlated, more real-time and precise information can be obtained, which can promote the development of cell biology. In the most present studies,

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the microelectrodes used for impedance monitoring are made of noble metals, such as gold. Gold microelectrodes have good conductivity, but little transparency, leading to the impossibility of cell morphology observation during the impedance monitoring. While, the utilization of indium tin oxide (ITO) microelectrodes can overcome this drawback, due to the optical permeability. Besides, ITO microelectrodes have many other advantages, such as biocompability and high stability, and has been considered as an ideal material for cell research [34]–[37].

In this study, SH-SY5Y cell adhesion is real-time monitored by impedance on ITO microelectrode arrays. Due to the transparency of microelectrodes, the changes of cell activities can be observed with the microscope simultaneously, when the cell impedance monitoring proceeds. The effect of As_2O_3 on cell adhesion is also evaluated by impedance measurement. Three concentrations of As_2O_3 are selected to perform the treatment and the impedance responses are continuously recorded. The cell survival rate is determined after the treatment of As_2O_3. Our studies may offer an effective way for the research of cytotoxicity.

II. Material and Methods

A. Chemicals and Materials

Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA, phosphate buffer saline (PBS, pH=7.4), and penicillin/streptomycin (P/S) were obtained from Gibco (Grand Island, NY). Arsenic trioxide (As_2O_3) and trypan blue solution (0.4%) were purchased from Sigma (St. Louis, MO). The solutions were prepared with deionized water (DI, 18.2 MΩ·cm). The reagents for photolithography, including photoresist tackifier (RZN-6200), positive photoresist (RZJ-304) and positive photoresist developer (RZX-3038) were obtained from Ruihong Corp. (Suzhou, China).

The plastic monitoring chamber and platinum wire were purchased from CHI Crop (China) and Goodfellow (France), respectively. ITO glass was purchased from Zhuhai Kaivo Electronic Co. Ltd (China). The ITO layer is ~180 nm thick with low electrical resistance (10 $\Omega \cdot cm^{-2}$) and high transmittance (>83%).

B. Fabrication of ITO Microelectrode Arrays and Detection Device

ITO microelectrode arrays was patterned with photolithography, and the procedures of fabrication were as follows: Firstly, a glass slide was cleaned respectively with acetone, ethanol and deionized water and then dried with N_2. Secondly, photoresist tackifier and positive photoresist were spin-coated on the clean slide with 5 μm in thickness, successively. Then, the ITO substrate with 0.1 cm^2 in culture area was baked at 90 °C for 3 min, followed by the exposure to UV light through a photomask, which has 3×3 micropore arrays with 50 μm in diameter. After that, the photoresist was developed in the developer solution. Finally, the fabricated ITO microelectrode arrays were baked at 130 °C for 3 min to remove the solvent.

A plastic monitoring chamber was adhered reversibly on ITO microelectrode arrays and could be reused. As shown in Fig. 1, the device for cell impedance monitoring is consisted of ITO microelectrode arrays as working electrode, Pt wire as both of reference electrode and counter electrode, and the monitoring chamber. All electrodes were electrically linked to CHI 660C electrochemical workstation (Chenhua, Shanghai).

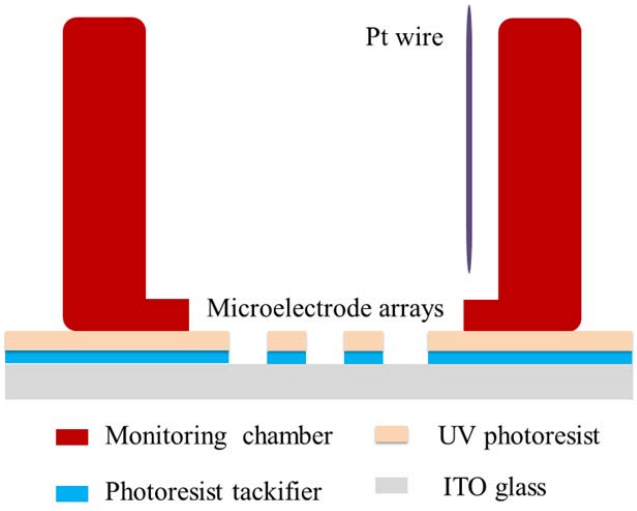

Fig. 1. Schematic diagram of the device for impedance monitoring.

C. SH-SY5Y Cell Culture and the Treatment of As_2O_3

The human neuroblastoma SH-SY5Y cells, provided by the laboratory of Prof. Chonggang Yuan (School of Life Science, East China Normal University), were cultured at 37 °C in an atmosphere containing 5% CO_2 with DMEM, supplemented with 10% FBS and 1% P/S. After dissociation in a 0.25% trypsin-EDTA solution and centrifugation, cell number and viability were evaluated by direct cell counting with trypan blue staining. Then, the cells were resuspended in culture medium and seeded on the surface of microelectrodes with the density of 1×10^5 cells/mL. The culture time lasted for 12 h.

In order to study the effect of As_2O_3 on cell adhesion, after 3 h of cell seeding, As_2O_3 solution with three different concentrations (10 μmol/L, 20 μmol/L and 40 μmol/L) was added into the culture system, and 9 h later, the cell survival rates were determined with trypan blue.

D. Impedance Monitoring

The impedance monitoring was performed at 37 °C. After the electrodes were electrically linked, impedance spectra was recorded every 30 min to monitor the cell adhesion and the effect of As_2O_3 on cell adhesion. The frequency of impedance spectra ranges from 1 kHz to 100 kHz with an AC voltage of 10 mV. In this paper, cell index (CI) is used to express the impedance response, which is defined by Equation 1:

$$CI = \frac{Z_{cell}}{Z_0} - 1 \tag{1}$$

where Z_0 and Z_{cell} are the impedance without and with cells on ITO microelectrode arrays collected at 25 kHz, respectively.

For the control experiment, only culture medium was put in the plastic monitoring chamber and the other conditions remained unchanged. All experiments were repeated for

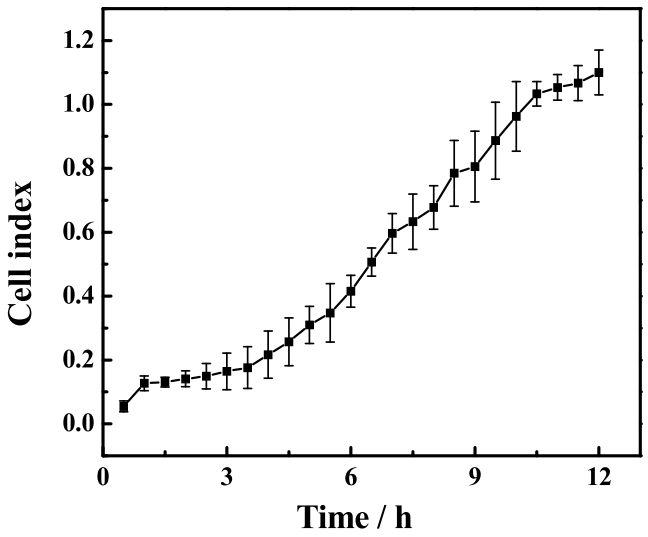

Fig. 2. Cell index curve of SH-SY5Y cells with the initial density of 1×10^5 cells/mL cultured on ITO microelectrode arrays for 12 h.

three times and data were expressed in the figures as mean of three repeated results.

E. Microscopic Observation

Due to the transparency of ITO microelectrode arrays, they can be directly placed on the stage of the inverted microscope (Olympus IX51, Japan) for cell imaging with CCD camera. Microphotographs of cells were captured every 30 min during the cell adhesion and the treatment of As_2O_3 for the observation of cell activities.

III. Results and Discussion

A. Monitoring of Cell Adhesion

An impedance determination system was used here to monitor SH-SY5Y cell adhesion. By culturing SH-SY5Y cells on ITO microelectrode arrays, impedance signals were collected, and meanwhile, cell images were acquired, which are another important characteristic for cancer cell research. 1×10^5 cells/mL was selected as the cell seeding density, in order to ensure that the entire electrode surface was covered by SH-SY5Y cells within the culture time of 12 h. As indicated in Fig. 2, in the first 1 h, the cell index increases quickly due to the subsidence of suspended cells and the initial attachment to the microelectrode surface. In the later 3 h, the cell index shows a relatively slow increase, because the cells finish the settling and begin to spread over the microelectrodes, resulting in the gradually reduced active area of electrodes. Then, the cells continue the spreading process, which contributes to a change of cell index with higher rate.

Fig. 3 present the microphotographs and the corresponding numbers of SH-SY5Y cells after cell seeding for 1 h, 4 h, 8 h and 12 h. It can be observed that the cells have attached the microelectrodes at 1 h, but most of them are still in the round shape. In the next 3 h, the cells launch the spreading on the microelectrodes. At 8 h of cell seeding, the cells have extended gradually on the microelectrode. Another 4 h later, cell monolayer is formed and the microelectrode surface is

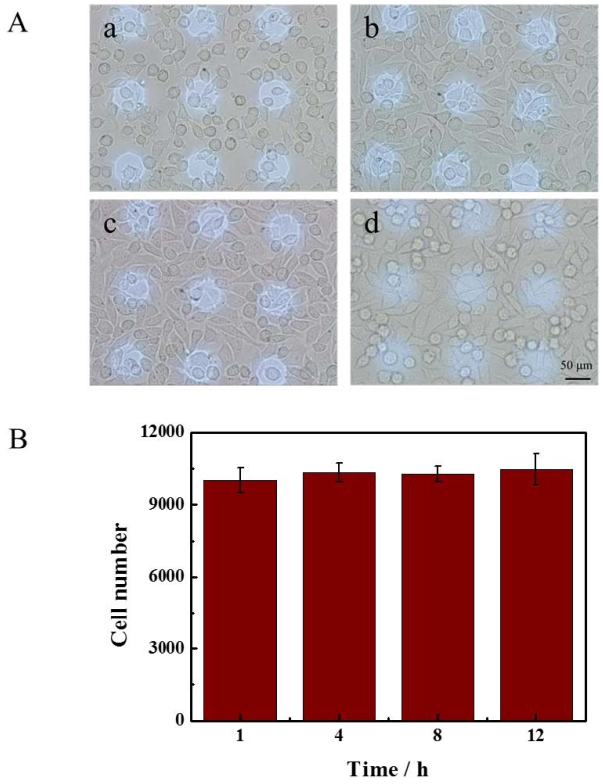

Fig. 3. Microphotographs (A) and numbers (B) of SH-SY5Y cells cultured on ITO microelectrode arrays with the initial density of 1×10^5 cells/mL for 1 h (a), 4 h (b), 8 h (c) and 12 h (d).

covered by the cells. During the period of monitoring, the cell number keeps almost constant. Correlating further the cell microphotographs to the cell index curve in Fig. 2, it is found that the changes of cell index could be interpreted with these microphotographs as powerful proof: The cell index increases fast because of the adherent cell at an increasing number on the microelectrode surface in the first 1 h. The followed initial spreading of cells contribute to slower increase in the cell index. While, the increasing rate of cell index is raised, caused by the cell spreading and gradual covering of microelectrodes. The above results indicate that the cell impedance is a reliable method to achieve the kinetics research of cell activities.

B. Effect of As_2O_3 on Cell Adhesion

As_2O_3 is a natural substance. Previous studies have certificated that As_2O_3 can induce the apoptosis of various NB cell lines. In order to study the effect of As_2O_3 on the adhesion of SH-SY5Y cells, As_2O_3 at different concentrations was introduced into the cell culture system and its effect on cell adhesion was monitored by impedance on the microelectrode arrays during 9 h of treatment time. The final concentrations of As_2O_3 were fixed at 10, 20 and 40 μmol/L. Every cell index curve presents the kinetic information of cell responses to As_2O_3 at different concentrations in details. From Fig. 4, it could be seen that the impedance responses to 10 μmol/L As_2O_3 are almost the same as the control without As_2O_3 treatment. In other words, 10 μmol/L As_2O_3 almost has no impact on cell adhesion. When the concentration of As_2O_3 increases to 20 μmol/L, the impedance decreases slightly in

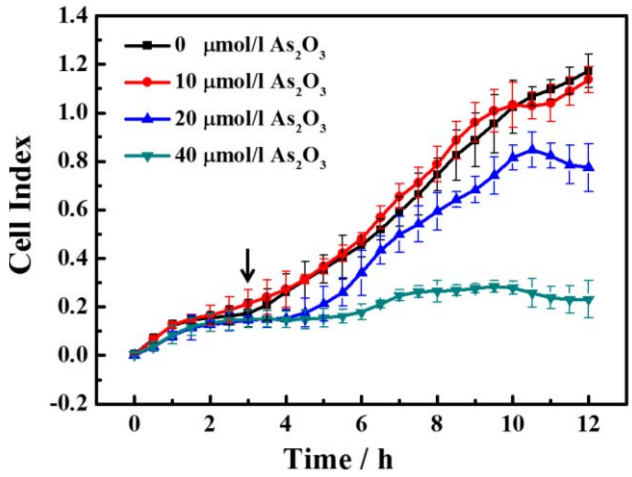

Fig. 4. Cell index curves of SH-SY5Y cells treated with As_2O_3 at different concentrations: 0 μmol/L, 10 μmol/L, 20 μmol/L and 40 μmol/L. The introducing time of As_2O_3 into the culture medium is labeled by arrow.

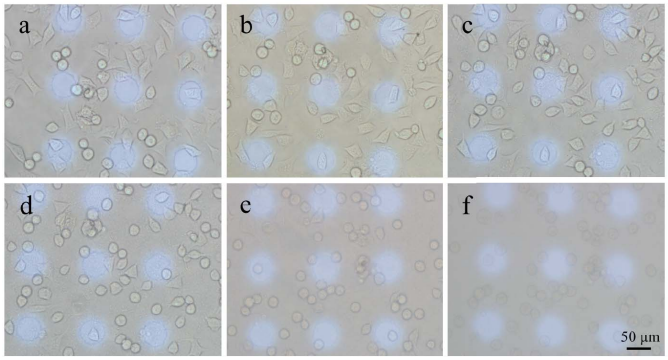

Fig. 5. The microphotographs of SH-SY5Y cells treated with 40 μmol/L As_2O_3 for 1 h (a), 2 h (b), 3 h (c), 4 h (d), 6 h (e) and 8 h (f).

the previous 7 h compared to the control. However, 7 h later, the signals have a sharp decline, indicating that the effect of As_2O_3 on cell adhesion begins to be shown obviously at that time. When the concentration of As_2O_3 increases continuously to 40 μmol/L, we can observe that the impedance increases much slowly and after 6 h of treatment, decreased impedance responses appear, probably due to the destruction of cell adhesion by As_2O_3. These results indicated that As_2O_3 at low concentrations required relative longer time to express its effect, while, As_2O_3 at high concentrations could affect the cell adhesion quickly. Moreover, it is proved that the cell impedance can monitor the cell responses to As_2O_3 treatment, providing real-time kinetic information without labeling during the entire experimental period.

The microphotographs of SH-SY5Y cells cultured in the medium with 40 μmol/L As_2O_3 were captured in the treatment process. Fig. 5 illustrates that until 3 h after introducing 40 μmol/L As_2O_3, cell activities has no obvious changes, while, after 4 h of treatment, part of the cells became spherical and a few of the cells were suspended in the medium. 8 h later, almost all of the cells are detached from microelectrode surface and changed to the round shape. Linking the cell microphotographs to the cell index curve in Fig. 4, it is found that changes of impedance signals are largely consistent with cell activities observed in the microphotographs: in the first 6 h of treatment, the impedance responses have no significant increase, which is contributed to the fact that the cells shrink to the round shape. Then, the cell index shows an obvious decline and the reason is that the cells are almost separated from the microelectrode surface and changed to the suspension status. More importantly, the cell index curve presents the effect of 40 μmol/L As_2O_3 on cell adhesion immediately after its addition to the culture medium, while, the same effect could be observed from microphotographs after 4 h of treatment. In other words, cell impedance could detect the effect of 40 μmol As_2O_3 on cell adhesion 3-4 h earlier than the microscopic observation, indicating that the cell impedance is more sensitive to the changes of cell activities induced by As_2O_3 and the information of the related effect can be obtained from impedance monitoring prior to the imaging analysis.

The cell survival rates determined after the treatment of 10, 20 and 40 μmol/L As_2O_3 for 9 h, are 94±3.9%, 90±2.9% and 80±5.0%, respectively. Without As_2O_3 treatment, the corresponding value is 96±3.6%. The results show that significant difference on cell survival rate can be seen with the treatment of 40 μmol/L As_2O_3, which is correlated to the decreased impedance response and the spherical and suspended cell status at the final of the treatment. Under such conditions, cell adhesion is greatly affected, so that the cells cannot continue the function of division etc., and the cell survival rate could not be maintained.

IV. Conclusions

In this paper, we have developed a cell impedance method for continuous and real-time monitoring of SH-SY5Y cell adhesion and its responses to the treatment of As_2O_3 on ITO microelectrode arrays. With the cell adhesion on electrode surface, the impedance increases gradually. However, the increasing rate is different, when the cells are in different stages of adhesion process. Cell activities can be observed with the microscope simultaneously and its changes are in good correlation with that of impedance. Moreover, the effect of As_2O_3 on cell adhesion is studied. In the selected period, 40 μmol/L As_2O_3 can significantly affect cell adhesion after 9 h of treatment, leading to the spherical and suspended cell status and obviously declined cell survival rate, which are correlated well with the decrease in impedance responses. The cell impedance can provide the label-free and real-time kinetic information of cell activities. Furthermore, it is more sensitive to the changes of cell activity and it can determine these changes much earlier than microscopic analysis. Our research is sure to promote the research of As_2O_3 effect on neuroblastoma and cancer therapy.

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