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Electric cell-substrate impedance sensing (ECIS) for profiling cytotoxicity of cigarette smoke

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ABSTRACT

Cigarette smoke contains abundant toxicants, and profiling its cytotoxicity represents a critical topic. In this study, cell-substrate impedance sensing (ECIS) was used to measure the cytotoxicity of 4-(methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK), nicotine, and the total particle material (TPM) of high-tar and low-tar cigarettes on CHO-K1 cells. Normalized impedance values at 3174 Hz were collected with microscopic imaging as an assistant, showing the dynamics of cell damage and the ability for cell self-recovery. The NI determination of the four toxicants indicated that, as the concentration of toxicants increased, cigarette smoke produced more intense toxic effects on the cells, and the ability of cell self-recovery worsened until there was permanent damage to the cells and the cells eventually died. Furthermore, the survival rate of the cells was obtained during treatment. NRU assays as a comparison were developed for evaluating the cytotoxicity by calculating the IC_{50}. Both methods showed that the cytotoxicity decreased in the following order: the TPM of high-tar cigarettes, TPM of low-tar cigarettes, nicotine and NNK. The sensitivity of the ECIS method was higher. Our work provides a useful and convenient approach for determining the cytotoxicity of cigarettes in a real-time, label-free manner, contributing to the development of low-toxicity cigarettes.

1. Introduction

Cigarette smoke contains > 5000 kinds of chemical substances [1], in which many toxicants have been found, such as 4-(methylnitrosoamino)-1-(3-pyridinyl)-1-butanone, nicotine, *N*-nitrosamines, tar, and cyclic aromatic hydrocarbons [2,3]. These toxicants can cause DNA damage, transformation of cytogenetic information, a variety of cancers, and so on [4–6]. Thus, research on detecting and evaluating the toxicity of cigarette smoke has attracted much attention and become an extremely urgent subject to investigate in terms of its influence on human health and the production of less harmful cigarettes.

In vitro toxicology research for evaluating the activity of cigarette smoke has been mainly realized by cytotoxicity tests, bacterial mutagenesis analysis and the micronucleus assay [7], in which cytotoxicity assays are the most sensitive and effective to test hazardous compounds, reflecting the biological effects of cigarette smoke [8–10]. Traditional cytotoxicity assays include neutral red uptake, MTT (thiazolyl blue) colorimetric method, and Cell Counting Kit-8 (CCK-8) [11–14]. Among them, neutral red uptake, which measures the integrity of the cell membrane and lysosomes has higher sensitivity when cells are exposed to toxicants, and thus is frequently used for evaluating the toxicology of cigarette smoke [15]. However, these methods require complicated operation steps and, more importantly, they are based on end-point detection, which cannot be used as a real-time monitor of the response of cells to toxicants.

To solve the above problem, electric cell-substrate impedance sensing (ECIS), as a non-invasive, label-free and real-time detection method for the monitoring of cell behaviours, has attracted wide attention. ECIS can overcome the limitations of conventional end-point tests in vitro [16–20]. When the cells attach and spread on the electrode, the current is physically impeded, resulting in an increase of impedance. Thus, the ECIS response could reflect cell morphology, movement, activity, etc. Giaever and Keese first used an ECIS device in 1984 to monitor mammalian fibroblasts on a small gold electrode [21]. At present, ECIS has been widely employed for the measurement of cellular behaviours [22,23], wound healing [24], identification of cancer cells [25] and cytotoxicity [26–30]. Studies have reported that impedance is used to evaluate the cytotoxicity of abundant substances, including metal nanoparticles [26,27], cancer drugs [28], marine pollutants [29], viruses [30], and so on. While there have been a few reports on the application of ECIS in the evaluation of tobacco toxicity [31,32], there are little or no reports on its use in monitoring the ability of cells to recover after poisoning. The ability of cells to recover could further explain the extent of the toxic effect of cigarette smoke on the

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cells.

The purpose of this work is to establish a convenient ECIS system to provide real-time and dynamic information during treatment with cigarette smoke to evaluate its cytotoxicity. A homemade ECIS device has been fabricated and utilized to profile the cytotoxicity of 4-(methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK), nicotine and total particle material (TPM) of high-tar and low-tar cigarette smoke on Chinese hamster ovary cells (CHO-K1). Moreover, ECIS was used to assess the self-recovery ability of cells after the treatment with tobacco toxicants. The cells were imaged simultaneously for observation of cell morphology. Cell survival rates and IC_{50} values were further calculated from the signals of ECIS and compared with those from conventional neutral red uptake assays. Our work offers a real-time, label-free and continuous monitoring method to profile the cytotoxicity of cigarettes, potentially promoting the development of low toxicity cigarettes.

2. Materials and methods

2.1. Materials and reagents

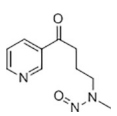
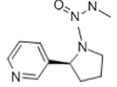
Polytetrafluoroethylene (PTFE) was provided by Shanghai Plastics Research Institute (China). Polydimethylsiloxane (PDMS, Sylgard 184) was purchased from Dow Corning (Shanghai, China). ITO (Indium Tin Oxide) glass was provided by Zhuhai Kaivo Optoelectronic Technology Co. Ltd., (China). Pt gauze was provided by Wuhan Gaoss Union Technology Co. Ltd., (China). Dimethylsulfoxide (DMSO) was obtained from the Sigma-Aldrich Company. Neutral red was obtained from Sangon Biological Engineering Co. Ltd. Dulbecco's modified eagle medium, Ham's F-12 (DMEM/F12), foetal bovine serum (FBS), penicillin/streptomycin (P/S), trypsin/EDTA solution, and trypan blue solution were purchased from Gibco (Shanghai, China). 4-(Methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK) and nicotine were purchased from Macklin Technology Co. Ltd. (Shanghai, China). A brief description is given in Table 1. Total particle material (TPM) of high-tar and low-tar cigarettes from cigarette smoke were provided by Shanghai Tobacco Group Co. Ltd., (Shanghai, China). The Chinese hamster ovary (CHO-K1) cells were purchased from the Chinese Academy of Sciences (Shanghai, China). All solutions were prepared with deionized water (DI, 18.2 $M\Omega \cdot cm^{-1}$).

2.2. Fabrication and assembly of the ECIS device

The ECIS device was developed to achieve real-time cell impedance monitoring and simultaneous cell imaging. As illustrated in Fig. 1, the device was fabricated on sterilized ITO glass [33–36]. ITO glass was used as the working electrode (WE) due to its good electrical conductivity and transparency, which could facilitate the collection of sensitive impedance signals and microscopic observation. Pt gauze with large area and good stability was employed as the counter electrode (CE) and reference electrode (RE). The inverted trapezoidal chamber and baseboard was made with PTFE due to its non-toxicity. The cell chamber was sealed on the ITO glass with a PDMS seal ring with biocompatibility, thus improving the tightness of the ECIS device. The effective area of the CE/RE and the WE were fixed at 7.06 cm^2 and 0.28 cm^2, respectively. The ratio of 25 (CE/RE:WE) showed that the signal was mainly determined by cells attached on the working electrode surface, which could improve the detection sensitivity [37,38]. This device was assembled with screws, facilitating the replacement of WE and recycling of other components.

Before cell seeding, the device was cleaned ultrasonically with ethyl alcohol and deionized water for 30 min, followed by sterilization at 120 °C for 20 min and irradiation with ultraviolet light for 1 h.

Table 1
Two toxic components in cigarette smoke.

Sample	Molecular formula	CAS #	Standard purity (%)	Structure
NNK	$C_{10}H_{13}N_3O_2$	64091-91-4	99	
Nicotine	$C_{10}H_{14}N_2$	54-11-5	99	

2.3. Cell cultures

The CHO-K1 cells were cultured in Dulbecco's modified Eagle medium (DMEM), Ham's F12 (1:1) containing 10% FBS and 1% (P/S), in a humidified incubator (NuAire, USA) in 5% CO_2 and 95% air at 37 °C. After reaching 80% confluence, cells were detached with 0.25% trypsin/EDTA, followed by centrifugation (800 rpm, 5 min). After removing the trypsin solution, cells were resuspended in culture medium. The cell density was determined by trypan blue staining and counting, and then the cells were seeded into the ECIS device.

2.4. Collection of total particle material (TPM) in cigarette smoke

The TPM of two commercial brands of cigarettes with a brief description in Table 2 was collected as reported with minor modifications [39]. The cigarettes were combusted with 35 mL of puff volume and 2 s of puff duration at a rate of 1 times/min using a Borgwaldt 20-port rotary smoking machine (provided by Shanghai Tobacco Group Co. Ltd.). TPM collected in Cambridge fitters was extracted with 10 mL of methanol, which was then removed by rotary evaporation. Afterwards, the TPM was re-dissolved in DMSO at a concentration of 30 mg/mL and stored at −70 °C until assayed.

2.5. Cytotoxicity evaluation by ECIS

NNK, nicotine and TPM from two commercial brands of tobacco (Hard Chunghwa, Jose Sent) were employed as toxics to treat cells for evaluating the cytotoxicity of cigarette smoke. NNK and nicotine were dissolved respectively in DMEM/F12 supplemented with 10% FBS and 1% P/S to achieve stocks at a concentration of 10 mg/mL. First, the impedance signals without cells were measured every 2 h in the frequency range from 1 Hz to 100 kHz with a signal amplitude of 10 mV using an electrochemical station IGS4030 (Guangzhou Ingsens Sensor Technology Co., LTD., China), obtaining the value of Z_0. Then, the medium was removed, and 4 mL of cell suspension with a density of 20,000 cells/cm^2 was introduced, followed by the collection of impedance signals every 2 h. After 24 h, the cells were treated with toxicants dissolved in fresh medium (NNK: 1.0, 2.5, 3.0, 4.0 and 6.0 mg/mL; nicotine: 0.1, 0.5, 1.0, 2.0 and 4.0 mg/mL; TPM (Chunghwa or Jose Sent): 12.5, 25, 50, 75 and 100 μg/mL). The impedance responses were continuously monitored every 2 h for another 24 h. After that, the toxicants were removed and replaced by fresh culture medium, and the ECIS detection was carried on at 2 h intervals in the next 24 h to observe the ability of cells to recover. Meanwhile, the cells were imaged every 6 h during the whole process of ECIS determination with an inverted fluorescence microscope (Olympus IX51, Japan).

2.6. NRU assay

The contrast experiments were determined by the NRU assay. Briefly, the cells were seeded in the 96-well plates at a density of 1750 cells/cm^2 and cultured for 24 h. Then, the cells were exposed to the four toxicants at concentrations corresponding to ECIS monitoring for 24 h, respectively. After the removal of toxicants, cells were brought into the FBS-free medium with 10% neutral red solution for 3 h in the incubator. Afterwards, the neutral red solution was removed and

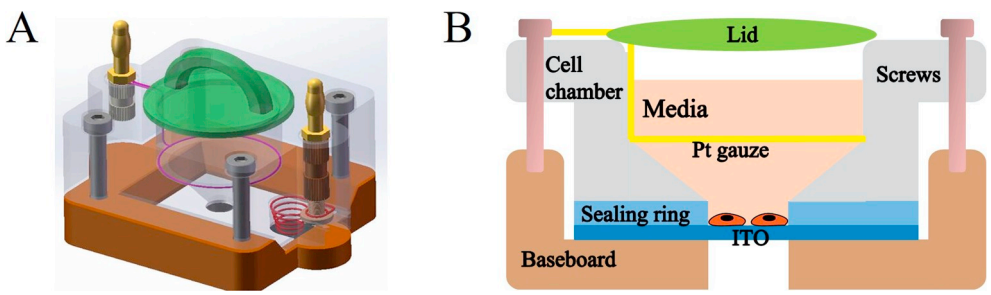


Fig. 1. (A) 3D illustration of the ECIS device with the ITO microelectrode as the working electrode and Pt gauze as the reference and auxiliary electrode. (B) Side view of the ECIS device assembly showing the main components, including the cell chamber, Pt gauze, PDMS seal ring, ITO glass and baseboard.

Table 2
Two brands of cigarettes.

Brand	Description	Tar (mg)	CO (mg)	Nicotine (mg)
Chunghwa	Virginia	11	11	1.0
Double happiness (Hepai)	Virginia	5	8	0.5

replaced by 200 μL of 50% ethanol, 49% water, and 1% glacial acetic acid. The 96-well plates were shaken immediately for 10 min using the shaker in order to completely lyse the cells. Finally, the absorbance of the solution in each well was measured at 540 nm with a microplate reader. Each experiment was repeated at least three times.

3. Results and discussion

3.1. Cytotoxicity evaluation by ECIS

The ECIS measurement was performed to evaluate the cytotoxicity of tobacco and self-recovery ability of cells after treatment. The previous research work has verified that Chinese hamster ovary cells (CHO-K1) with the advantages of rapid reproduction and easy cultivation, are more sensitive to cigarette smoke with stable test results compared with other cell lines [31,40]. Therefore, this cell type was selected as a model for cytotoxicity assays. ECIS is a frequency-dependent measurement. When the frequency $f \leq 1$ Hz, the cells are poor conductors, which will affect the conductivity of the entire system. When the frequency $f > 100$ kHz, the measured impedance mainly reflects the change of the medium due to the current penetrating the cell membrane. Therefore, the frequency spectroscopy plots of impedance were recorded in the range of 1 Hz–100 kHz with a signal amplitude of 10 mV [41]. The availability of the ECIS device was first investigated in 0.1 mol/L and 1 mol/L NaCl solution during a period of 48 h, respectively. As shown in Fig. S1, the both impedance curves were basically level with the deviations of ± 0.3 at 3174 Hz within 48 h, exhibiting the availability of the device for cellular culture and toxicity evaluation. In the toxicity assay, the initial cell density was fixed at 20,000 cells/cm^2, which allowed cells in the exponential growth phase at the time of adding toxicants. Fig. 2 displays the continuously increased impedance responses during 72 h of culture without toxicant treatment. Obviously, the largest difference appears at 3174 Hz. Hence, 3174 Hz was selected as the characteristic frequency for CHO-K1 cells in the following impedance measurements to obtain the highest sensitivity. To eliminate the effect of culture medium, the impedance of cells were calibrated by the impedance of culture medium without cells, obtaining the normalized impedance (NI), which could be expressed as the following equation [42]:

$$NI = \frac{Z_{cell} - Z_{cell-free}}{Z_{cell-free}} \quad (1)$$

where Z_{cell} and $Z_{cell\text{-}free}$ represent the impedance with and without cells in the device at the same conditions. The corresponding equivalent

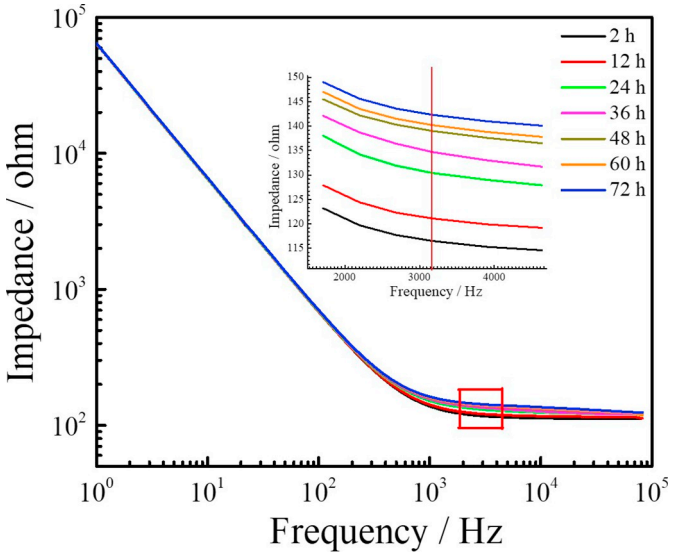


Fig. 2. Bode plots for the growth of CHO-K1 cells (without toxicants) within 72 h on the ITO microelectrodes.

circuits were introduced to illustrate the electrical properties of impedance device. When no cells were presented, the impedance of the system can be represented by an equivalent circuit with two parameters: the solution resistance (R_s) and the double layer capacitance of the electrodes (C_{dl}) (Fig. S2A). When the cells were cultured on the working electrode, the impedance could be modeled as a resistance (R_c) and a capacitance (C_c) related to the cell viability and growth besides the impedance contributions from the R_s and C_d (Fig. S2B).

NNK from the aerosol phase and nicotine from the particle phase are representative carcinogens of cigarette smoke. The former is a strong carcinogen in animals, and the latter can cause increased cardiac blood transfusion blood pressure and peripheral vascular contraction. TPM causes more harm to the human body due to its complex composition. Therefore, the toxicities of two pure substances and the TPM from two commercial brands of cigarettes were examined based on NI changes in the cell population. For the control experiments, fresh culture medium was used instead of toxicants. As shown in Fig. 3A, during the period of NNK treatment, NI values continued to rise less slowly at 1.0 mg/mL than the control responses. When the concentration increased to 2.5 mg/mL, the cells exhibited the same profile at the initial 8 h of exposure as those treated with 1.0 mg/mL NNK. After 8 h, the NI values grew more slowly. Increasing the NNK concentration to 3.0 mg/mL caused the NI values to be basically unchanged. Treatment with 4.0 mg/mL and 6.0 mg/mL NNK caused NI values drop sharply to nearly zero, which indicates that NNK has a stronger inhibitory effect on the cell growth as its concentration increases. After the cells were exposed to toxicants for 24 h, fresh medium was introduced in the cell culture chambers. Clearly, the cells treated with NNK at the

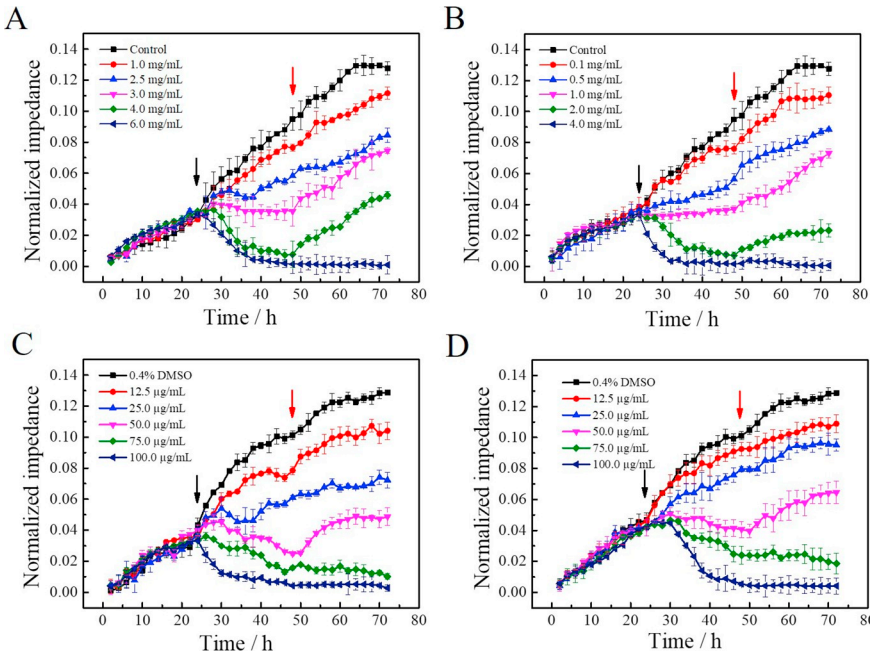


Fig. 3. Real-time monitoring of the impedance response at 3174 Hz to (A) NNK, (B) nicotine, (C) TPM of high-tar cigarettes and (D) TPM of low-tar cigarettes. All the data are represented by means ± SD, n = 3.

concentrations other than 6.0 mg/mL were able to continue growing with a similar slope compared with the untreated cells, revealing that NNK at a lower dose cannot completely damage the cells, which could continue to proliferate with renewal of the culture medium. Meanwhile, treatment with 6.0 mg/mL NNK did not generate increased NI values, which maintained values near zero, showing that NNK at this concentration can kill all the cells and produce permanent damage. Fig. 3B displays the NI values of CHO-K1 cells exposed to nicotine at different concentrations. In the presence of 0.1 mg/mL nicotine in the medium, smaller NI values were found compared with the control cultures without toxicant treatment after 6 h of exposure, which indicates that nicotine does not have a quick toxic effect on the target cells at the beginning of exposure. Increasing the concentration of nicotine to 0.5 mg/mL caused the NI values to grow more slowly. Meanwhile, 1.0 mg/mL nicotine produced almost constant NI values. When the concentration of nicotine was increased to 2.0 mg/mL and 4.0 mg/mL, NI values exhibited a decreasing trend, indicating more serious damage to cells. After renewal of the culture medium, NI values presented a continuous upward trend, showing that the cells were able to continue growth with the nicotine at concentrations other than 4.0 mg/mL. Only the treatment with 4.0 mg/mL of nicotine did not generate increased NI values, indicating that nicotine at this concentration can kill all cells and produce permanent damage. Fig. 3C and D show the NI values of cells cultivated with TPM from two cigarettes containing high tar and low tar, respectively. To eliminate the effect of DMSO on ECIS response, the NI values obtained in the medium with 0.4% DMSO was used as a reference. It has been reported that when its concentration is higher than 0.5%, DMSO can present its toxicity on tested cells. From the NI vales, it could be observed that the increasing rate with the treatment of TPM from high-tar cigarettes was significantly smaller than the low-tar cigarettes at the same concentration. Moreover, 75 μg/mL and 100 μg/mL of TPM from both kinds of cigarettes basically killed the cells, which no longer resumed growth after growth medium replacement.

Images of cells were captured simultaneously along with the collection of ECIS signals (Fig. S3). Obviously, the untreated cells were uniformly arranged with basically the same size and clear outline. While, after exposure to NNK for 24 h, the cell number declined with rising concentration and the cell morphology also changed with cell wrinkling and contour blurring. 6 mg/mL NNK led to the death of most cells and the appearance of substantial amounts of cell debris. After the replacement with fresh culture medium, the cell number did not increase any more or even decreased with the treatment of 6 mg/mL NNK, while the cells treated with NNK at other concentrations were obviously able to proliferate and the outline of cells gradually became clearer. The control cells displayed normal proliferation. The same applies to the treatment with nicotine. TPM could rapidly induce cell death, thereby reducing the numbers of cells and changing the morphology. After the replacement of culture medium, the cells activated their own recovery system. However, the cells treated with TPM at 75.0 μg/mL and 100.0 μg/mL were greatly damaged and could not recover. These phenomena are more obvious in the cells treated with the high-tar cigarette. The microscopic observations match well with the ECIS determinations, indicating that ECIS can realize continuous data collection to evaluate cytotoxicity.

3.2. Determination of viability and IC_{50}

To further quantitatively assess the cytotoxicity of cigarette smoke, the cell survival rates were determined by ECIS and IC_{50} values were calculated. Due to the good linear correlation between impedance responses and cell numbers [43], the cells viability ratio could be calculated using Eq. (2) [29]:

$$\text{Viability ratio} = \frac{NI_{\text{treat}-t}}{NI_{\text{control}-t}} \times 100\% \quad (2)$$

where $NI_{\text{treat-t}}$ and $NI_{\text{control-t}}$ are the NI values at 3174 Hz of the treated and untreated cells at a given time, respectively. Meanwhile, the neutral red uptake (NRU) assay was employed to evaluate the cytotoxicity of each individual toxicant. NRU is a traditional method of detecting cytotoxicity, presenting high sensitivity relative to other conventional methods. The cell viability determined by the NRU assay was defined with Eq. (3) [44]:

$$\text{Viability ratio} = \frac{As - Ab}{\text{Ac} - \text{Ab}} \times 100\% \quad (3)$$

where *As* and *Ac* are the absorbance values at 540 nm of the treated and untreated cells at a given time, respectively. *Ab* is a control assay without cells and toxicants. Fig. 4 shows the cell viability ratios

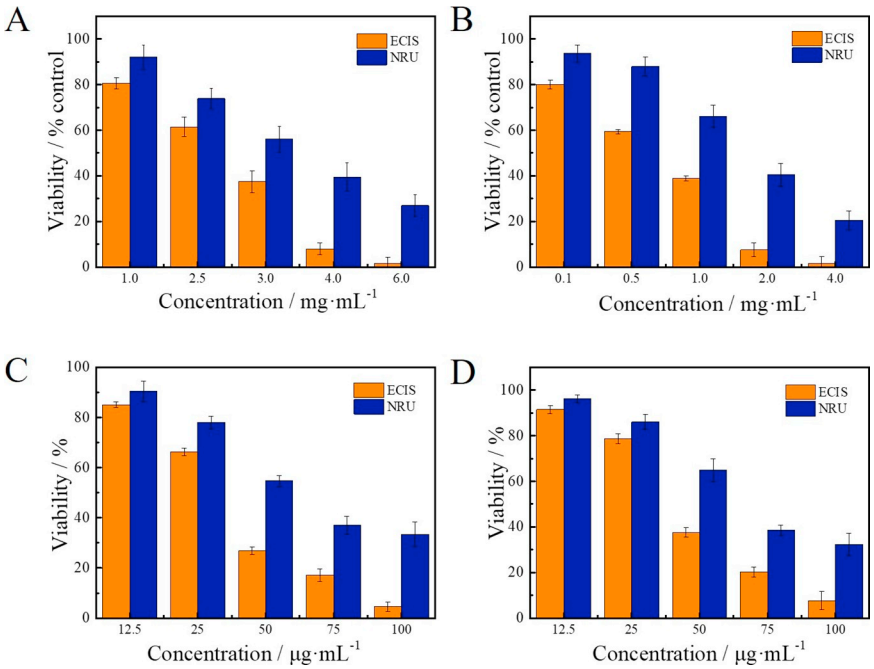


Fig. 4. Cell viabilities after treatment with (A) NNK, (B) nicotine, (C) TPM of high-tar cigarettes and (D) TPM of low-tar cigarettes for 24 h determined by the ECIS (NI values) and the NRU assay (absorbance values). Error bars represent the standard error of n = 3 values.

determined by ECIS and the NRU assay. Obviously, the viability of treated cells is concentration-dependent. The higher the concentration of toxicants, the lower the viability. Moreover, the cells exposed to toxicant at a fixed concentration within 24 h exhibited a smaller cell viability value by ECIS than by the NRU assay, revealing that the ECIS method has higher sensitivity than the NRU assay in terms of cytotoxicity due to their different measurement mechanisms. In the NRU assay, the neutral red dye is absorbed by lysosomes of viable cells, followed by the dissolution in the solvent buffer including 1% glacial acetic acid, 49% water and 50% ethanol, and then the measurement at 540 nm with a microplate reader. Hence, cell viability determined by the NRU assay only depends on lysosome activity. On the other hand, ECIS is able to monitor the responses of whole cells to the treatment of toxicants, and any related changes of cells, including cell morphology, cell activity and cell number, could cause the variation of ECIS signals. Therefore, the ECIS method is proved to be more sensitive than NRU assay in detecting cytotoxicity. In other words, ECIS technology is superior to the neutral red uptake assay in detecting the cytotoxicity of smoke. IC_{50} values declare the drug concentration that declines the cell viability to 50%. It is a both time and dose dependent parameter. As shown in Fig. 5, when cells were exposed to these four toxicants for 24 h, the NI value and cell survival rate (NRU) decreased with the rise of their logarithmic concentrations. Based on the Growth/Sigmoidal fitting curves (OriginPro 9), the values of IC_{50} and coefficients of variation (CV) were obtained using the ECIS method and the NRU assay. Table 3 shows that the mean data of IC_{50} and CV values obtained by the ECIS method were smaller or similar to those obtained by the NRU assay, once again confirming that the ECIS method has higher sensitivity with better accuracy to the changes of cells induced by cigarette smoke. What is more, it could be found that the IC_{50} of TPM of high-tar cigarettes has the highest values, followed by the TPM of low-tar cigarettes, nicotine and NNK, indicating that the TPM of high-tar cigarettes has the highest toxicity on the cells, then the TPM of low-tar cigarettes, nicotine and NNK. The probable reason is that NNK is formed by nicotine nitration and mainly inhibits the growth of cells. Nicotine is a toxic alkaloid, damaging cell proliferation and inhibiting cell repair. TPM is composed of nearly 6000 chemical constituents due to incomplete combustion caused by high temperatures and lack of oxygen, thus presenting much greater cytotoxicity on cells. Moreover, the more the tar content, the greater the cytotoxicity. Hence, it is important to develop cigarettes with low-tar and nicotine, in which ECIS provides a potential approach to evaluate the cytotoxicity of cigarette smoke.

4. Conclusions

The work employed ECIS to assess the cytotoxicity of NNK, nicotine and TPM of high-tar and low-tar cigarettes from cigarette smoke on CHO-K1 cells and the ability of cell self-recovery after poisoning by the normalized impedance at 3174 Hz in a rapid, continuous and label-free manner. Within 24 h after introducing toxicants, the NI value increased more and more slowly with increasing toxicant concentration, until it decreased significantly, nearing zero. After the fresh medium was replaced, the NI values continued to increase within 24 h of the treatment with toxicants at lower concentrations, while the cells exposed to the toxicants at higher concentrations presented unchanged or even decreased NI values, showing that the toxicants at higher concentration generated stronger cytotoxicity and the cells had lower self-recovery ability to continue the proliferation. The simultaneously captured cell images further evidenced the feasibility of ECIS for use in the evaluation of the cytotoxicity of cigarette smoke. The cell viability rates and IC_{50} values were then obtained based on the NI values, comparing with those obtained with the NRU assays. Both methods revealed the cytotoxicity of, in decreasing order, the TPM of high-tar cigarettes, the TPM of low-tar cigarettes, nicotine and NNK. However, ECIS provided more sensitive results than the NRU assay. Therefore, it shows great potential to establish a real-time cytotoxicity evaluation system with high speed and sensitivity for cigarettes, especially for the development of low-tar and low-nicotine cigarettes.

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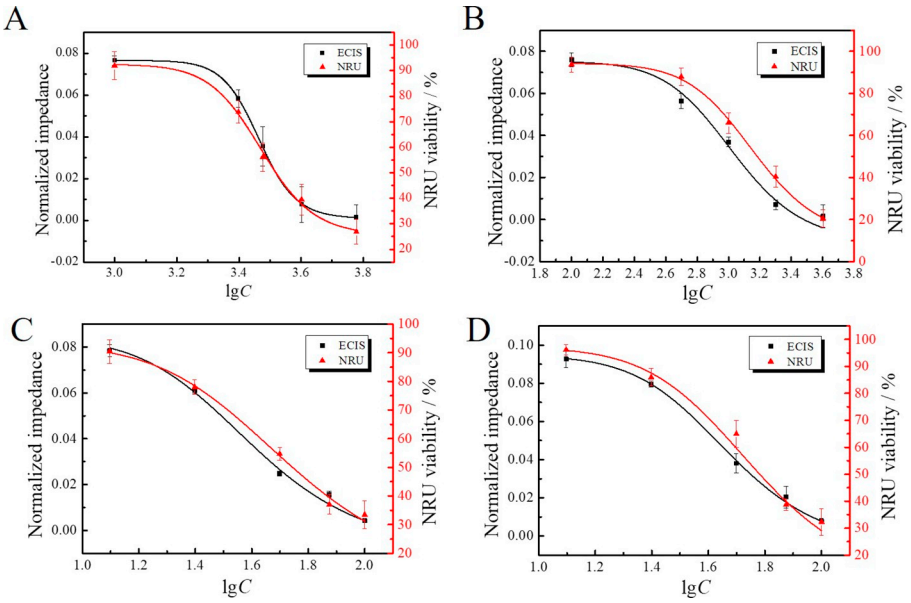

Fig. 5. Variation of NI values and NRU viabilities with increasing concentration. Cytotoxicity tests (n = 3) were performed using (A) NNK, (B) nicotine, (C) TPM of high-tar cigarettes and (D) TPM of low-tar cigarettes.

Table 3
Comparison of IC_{50} and CV values obtained by ECIS and NRU assays.

Sample	ECIS		NRU	
	IC_{50}	CV	IC_{50}	CV
NNK	2.923 ± 0.001 (mg/mL)	0.003	2.972 ± 0.001 (mg/mL)	0.003
Nicotine	1.074 ± 0.001 (mg/mL)	0.009	1.452 ± 0.001 (mg/mL)	0.007
TPM (Chunghwa)	39.934 ± 1.196 (μg/mL)	0.029	53.088 ± 2.070 (μg/mL)	0.032
TPM (Hepai)	45.708 ± 1.321 (μg/mL)	0.022	57.191 ± 1.531 (μg/mL)	0.027

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jelechem.2018.12.047.

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