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Evaluation of the cytotoxicity of cigarette smoke condensate by a cellular impedance biosensor

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ABSTRACT

In this study, a cytotoxicity assay was developed for profiling the cytotoxicity of cigarette smoke condensates (CSCs) base on a cellular impedance biosensor (CIB). Compared with the traditional in vitro cytotoxicity assays, this CIB-based method offered distinct advantages in real-time kinetic measurement which provided a comprehensive understanding of cellular responses for the entire duration of the experiment and prediction of the potential mechanism of action of a given treatment. The time-dependent cell response profiles provided valid evidences for optimization of cell number per well, cell quality control, and identification of the optimal time points for compound treatment and endpoint assays. According to the time dependent IC_{50} values, the CIB could provide dynamic information that can be used to identify maximum toxicity of cigarette smoke and reversibility of the toxic effects which are difficult to achieve by the endpoint assays. The comparative IC_{50} values indicated that the as-developed biosensor offered analytical results in good consistency with the commonly used NRU method. The features of the CIB-based cytotoxicity assay, such as no cell labeling, automatic detection, and easy operation, give this assay potential to become routine setting for evaluating the cytotoxicity of CSCs.

1. Introduction

Currently, tobacco smoking ranks as a major risk factor for premature death. The smoking of tobacco is a worldwide practice by over 1.1 billion people, especially in developed countries (International Agency for Research on Cancer, 2004). So, cigarette smoking is not going to disappear in the near future. The tobacco companies are actively pursuing different strategies to develop new cigarettes that might have the potential to decrease the risks associated with smoke (Andreoli et al., 2003). In the last years machine-measured tar yields of many cigarette brands have been reduced and the so-called 'low-tar' cigarettes have been introduced to address consumer concerns about health risks from smoking. However, whether the cigarette modifications eventually reduce the risk associated with smoking and how this reduction should be measured and characterized, calls for accurate toxicological evaluation. Hundreds of ingredients have been added into tobacco products such as liquorice to increase the smoothness of the smoke and menthol to provide distinctive, brand-specific flavor signature to the mainstream smoke (Carmines et al., 2005; Heck, 2010). The potential effects of ingredients during the process of cigarette burning should also be evaluated by in vitro assays (Baker et al., 2004).

As an important step in a number of pathological processes, cytotoxicity has been regarded as helpful in investigating the mechanisms of action of chemicals on cells and tissues and cell-based in vitro cytotoxicity tests have become essential techniques to rapidly assay hazardous compounds. Several in vitro cytotoxicity tests have been reported to determine the biological activity of CSCs (Tewes et al., 2003; Lou et al., 2009, 2010; Foy et al., 2004; Roemer et al., 2009). Eight in vitro assays have been evaluated to determine the most sensitive assays for assessing the cytotoxicity of CSCs in Chinese hamster ovary (CHO) cells. The results indicated the assays that measured membrane integrity was the most sensitive for short exposure times (1 h), whereas the neutral red uptake (NRU) and kenacid blue assays that measured total cell number were more sensitive for longer exposure times (24 h) (Putnam et al., 2002). Similar conclusions were reported by comparing the ability of four in vitro cytotoxicity assays in detecting cytotoxic

Abbreviations: ANOVA, Analysis of Variance; CHO, Chinese hamster ovary; CI, cell index; CIB, cellular impedance biosensor; CORESTA, cooperation center for scientific research relative to tobacco; CSCs, cigarette smoke condensates; CV, coefficient of variation; DMSO, dimethylsulfoxide; FBS, fetal bovine serum; NRU, neutral red uptake; TPM, total particle material.

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effects of cadmium chloride on hepatoma cell lines. The neutral red and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test assays were proved to be more sensitive in detecting cytotoxic events compared to the lactate dehydrogenase leakage and the protein assay (Fotakis and Timbrell, 2006). However, most of these conventional cytotoxicity tests utilize labeling or dye based endpoint detection to measure the number of cells dead or alive after exposure to chemicals and, therefore, need the cumbersome protocols or multiple washing steps. The influence of labeling or dye on cell behavior might be optimized to be subtle but indelible and the optical detecting methods are vulnerable to distortions because of compound interference. Moreover, the endpoint detection provides only cell viability at one given time point, but do not give information on the entire cellular dynamic process after adding toxicants.

As a complex mixture of more than 6000 compounds, tobacco smoke contains a diverse array of toxic chemicals, each of which has specific cytotoxicity (Smith and Hansch, 2000; Borgerding and Klus, 2005). Considering that the kinetics of cytotoxic compounds could differ dramatically between different compounds, it is important to monitor viability/toxicity continuously to analyze the cytotoxicity of complex tobacco smoke mixture. The reproducibility and accuracy of cytotoxicity assay should also be improved for profiling and differentiating the cytotoxicity of cigarette samples with different modifications. Hence, there is a strong requirement for label-free detection method with the competency of sensitive monitoring living cell behavior in real-time providing entire cellular dynamic process before and after exposure.

Impedance has recently received considerable attention as a label-free detection method of non-invasive cellular response profiling to screen the cytotoxicity of a number of chemicals (Boyd et al., 2008; Xing et al., 2005, 2006; Xia et al., 2008; Atienza et al., 2006; Opp et al., 2009) including drugs (Glamann and Hansen, 2006), environmental metal contaminants (Ceriotti et al., 2007; Xiao and Luong, 2005), and so on. Cells are seeded in microtiter plate whose bottom are integrated with gold interdigitated microelectrodes, so the impedance readout of microelectrodes can be utilized to non-invasively quantify cellular status in real-time, such as changes of ionic channels in cell membranes, the variations of cell membrane integrity and so on (Guo et al., 2006; Qiu et al., 2008). To our knowledge, there has been no report in existing literature concerning the use of cell-based impedance technique for evaluating the cytotoxicity of complex tobacco smoke mixture.

In this study, a cytotoxicity assay was proposed for profiling the cytotoxicity of CSCs based on the CIB which utilized non-invasive measurements allowing for time resolution and kinetic measurement. The CIB-based cytotoxicity assay was capable of showing distinct and important advantages over traditional endpoint assays by providing a comprehensive understanding of cellular response in real-time and prediction of the potential mechanism of action of a given treatment. The time-dependent cell response profiles offered valid evidences for optimization of cell number per well, cell quality control and identification of the optimal time points for compound treatment and endpoint assays. The comparative IC_{50} values indicated that the as-developed biosensor offered analytical results in good consistency with the commonly used NRU method. Moreover, dynamic monitoring with the CIB allowed for the calculation of optimized IC_{50} values in real time, which could be used to identify maximum toxicity of cigarette smoke and reversibility of the toxic effects. The results obtained revealed that the proposed CIB-based cytotoxicity assay could be a promising technique for evaluating the cytotoxicity of tobacco smoke condensate by virtue of no cell labeling, automatic detection, high throughput, and easy operation.

2. Materials and methods

2.1. Materials

Neutral red, glacial acetic acid, ethanol, formalin fixative and dimethylsulfoxide (DMSO) were purchased from the Sigma–Aldrich Company (St Louis, USA). Ham's F-12 medium, fetal bovine serum (FBS), L-glutamine, sodium bicarbonate and trypsin were obtained from GIBCO (Grand Island, USA).

2.2. Cell culture

The CHO cells were chosen due to their sensitivity in previous genotoxicity and cytotoxicity assays evaluating the activity of cigarette smoke (Bombick and Doolittle, 1995; Bombick et al., 1997). The CHO cell line (CHO-K1) was obtained from the American Type Culture Collection and maintained in Ham's F-12 medium supplemented with 10% FBS, 2 mM L-glutamine and 1.5 g/L sodium bicarbonate. Cells were grown at 37 °C in a humidified incubator maintained at 95% air and 5% CO_2. In preparation of CSCs exposure, CHO-K1 cells were trypsinized, centrifuged, and resuspended in the culture medium mentioned above and seeded in 96-well E-plates with optimized cell number/well.

2.3. Instrumentation

Cytotoxicity testing was performed using a CIB-based system (Roche xCELLigence system, Roche Diagnostics Australia, Castle Hill, NSW). The CIB was a disposable 96-well microtiter plate (E-plate 96) with gold interdigitated microelectrodes incorporated in the bottom. In experiment, the microtiter plate with cultured cells was mounted to a device station placed inside a CO_2 incubator. Electrical cables connected the device station to the sensor analyzer outside the incubator. For all impedance measurements, a sine-modulated ac potential of 20 mV was applied to the gold interdigitated microelectrodes. Impedance spectroscopy was performed in the frequency range from 1 Hz to 1 MHz using Electrochemical Impedance Spectroscopy program. Impedance of the interdigitated microelectrode at fixed frequency was measured at 25 kHz using the Capacitance-Potential program. The sensor analyzer could simultaneously monitor up to 96-well E-plates and the cell status in chosen well could be quantitatively measured using a dimensionless unit termed the cell index (CI) which was based on the impedance changes caused by cells interacting with the microelectrodes. The CI value at each time point was defined as Rn–Rb/15, where Rn was the cell-electrode impedance of the well with the cells, and Rb was the background impedance of the well with media alone.

The absorbance of neutral red in the NRU assay was measured by a microplate reader (Bio-Tek, USA). A Borgwaldt 20-port rotary smoking machine was utilized for cigarette smoking (RM-20H/CSR) (H. Borgwaldt, Hamburg, Germany).

2.4. Preparation of tobacco smoke condensate

Five commercial brands of cigarettes with different tar yields were chosen as sample A–E to demonstrate the capability of the CIB-based cytotoxicity assay. A brief description for these commercially cigarettes are given in Table 1. The CSCs of each brand were separately prepared according to protocols previously described with minor modifications (McAdam et al., 2011; Counts et al., 2004). Prior to smoking, all cigarettes were conditioned for at least 48 h and no more than 10 days in an environmental chamber maintained at a temperature of 22 ± 1 °C and 60 ± 3% relative humidity according to ISO standard 3402 (ISO, 1999). A sufficient number of cigarettes are smoked to trap 300–500 mg of total particle material (TPM) by a modified Borgwaldt 20-port rotary smoking machine equipped with an electrostatic precipitation trap according to the Federal Trade Commission conditions of a 35-mL puff for duration of 2 s, once every 60 s. Then the electrostatic precipitation tube ends were closed with end-caps after smoke collection and the trapped MS smoke particulate matter was rinsed into a vessel with 30 mL methanol. The methanol was evaporated under reduced pressure and then DMSO was added to achieve a final concentration of 100 mg TPM/mL. This primary stock was stored at −70 °C. Dilutions were made in complete cell culture medium using the thawed aliquots. DMSO was used as the solvent control in all assays.

2.5. Cell titration test

Firstly, proper electrical-contacts and the background impedance were measured after adding 50 μL media to the wells of E-plates 96. Subsequently, 100 μL of CHO-K1 cell suspension with adjusted cell numbers of 1000, 2500, 5000, 10,000, 15,000, 20,000 and 35,000 were seeded into the 50 μL medium containing wells. Wells containing 150 μL of culture medium only were used as blanks. Finally, the E-plate 96 was put into the incubator and the adhesion, growth and proliferation of the cells was monitored automatically by the CIB system. Each condition was performed in six replicate wells.

Table 1
A brief description about the nature of the five commercially cigarettes.

Sample	Description	Tar (mg)	CO (mg)	Nicotine (mg)
A	Blended type cigarette with charcoal filter	3	3	0.3
B	Blended type cigarette with charcoal filter	5	6	0.4
C	Virginia type cigarette	5	8	0.5
D	Virginia type cigarette	13	13	1.2
E	Blended type cigarette	8	10	0.7

2.6. Cytotoxicity assay using the CIB system and NRU assay

After subtracting background impedance with 50 μL of culture medium, 100 μL of cell suspension with optimal seeding concentration was added into the sensor wells. And then, the E-plates 96 were mounted on the CIB Station in the incubator. Cells attachment, spreading, and proliferation were monitored and visualized in real time using the CIB system. When cells were in the log growth phase, the cells were treated with tobacco smoke condensate at final concentration of 25, 50, 100, 150, 200, 250, 300 μg/mL and continuously monitored for up to 24 h. Controls included DMSO-only wells.

In the parallel experiments, the cytotoxicity of tobacco smoke condensate was tested using NRU assay according to literature with subtle modification (Lou et al., 2009). In brief, after CSCs exposure, the treatment medium was aspirated and replaced with 200 μL neutral red solution (50 μg/mL in culture medium without FBS). After a 3 h incubation period, the neutral red solution was removed and 200 μL of the 1% formalin fixative was added to each well for 1–2 min. The fixative was replaced immediately by 150 μL of the solvent solution (1% glacial acetic acid, 49% water, 50% ethanol) in each well. The 96-well culture plates were placed on a microplate shaker for 10 min, and then the absorbance of each well was measured at 540 nm on a microplate reader.

2.7. Data analysis

The IC_{50} values of CIB and NRU assay represented the concentration of CSCs producing 50% reduction of CI and the concentration of CSCs producing 50% reduction of neutral red uptake as compared to the negative control respectively. The IC_{50} values were obtained directly from the dose response curves, which has been previously described (Ceriotti et al., 2007).

Data was analyzed for significant differences ($p < 0.05$) between experimental groups by performing Analysis of Variance (ANOVA) with Tukey's post hoc multiple comparison test with *GraphPad Prism® software* (GraphPad Software Inc., San Diego, CA).

3. Results and discussion

3.1. Cell quantification

The CIB used for non-invasion cytotoxicity assay was based on impedance measurement of cells growing on microelectronic sensors integrated in wells of microtiter plates. In order to verify if the impedance readout of the electrode was quantitatively correlated with the CHO-K1 cell numbers, cell suspensions with different cell number were added into the wells of E-plates 96. Fig. 1A depicts the variation of CI values determined at different time after cell seeding and Fig. 1B shows the obtained CI values as a function of varying cell numbers titrated in the wells. It could be seen from Fig. 1A, the CI values of wells with high cell density represent non-linear growth when time is longer than 6 h, which might because of the cell growth and contact inhibition arising from high cell density. As shown in Fig. 1B, the CI values recorded within 6 h after cell seeding have good linear relationship with the cell numbers over a range of 1000–35,000 cells. Linearity of these calibration curves was rather good with correlation coefficients of above 0.99. In this period, the impedance response is proportional to the number of cells seeded in the wells, as well as the morphology of the cells and the quality of cell attachment. The coefficient of variation (CV) values of six replicate measurements within 6 h after cell seeding ranged from 1.6% to 14.8%, indicating that the CIB could provide comprehensive information regarding the biological status of cells, such as cell spreading, cell growth, and morphological changes.

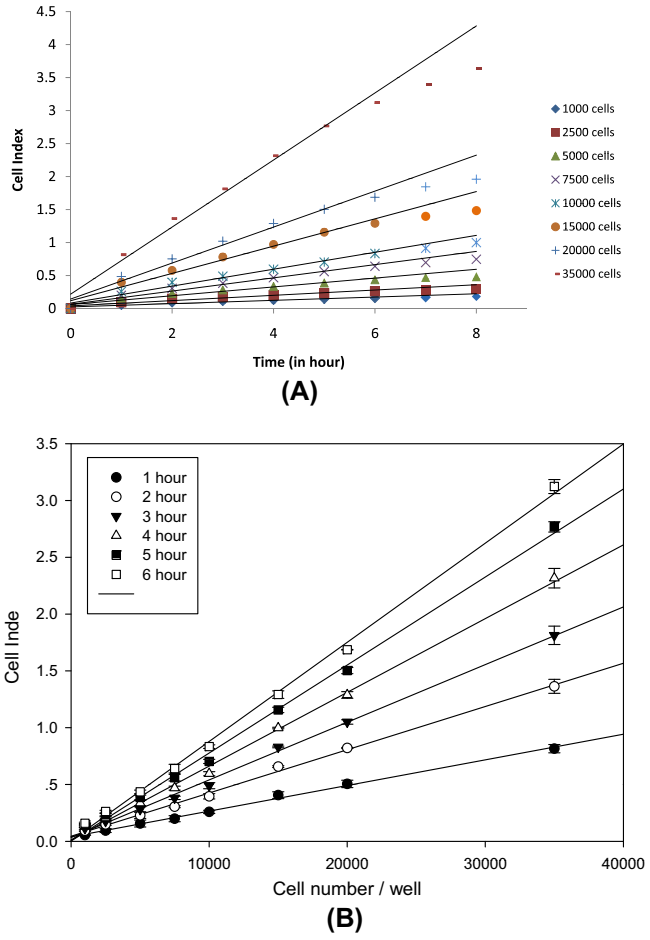

Fig. 1. Cell quantification by the CIB method. (A) Linearity of cell quantification based on CI values determined at different time after cell seeding; (B) linearity of cell quantification based on CI values as a function of varying cell numbers titrated in the wells.

3.2. Optimization of cell number/well and cell quality control

The cell seeding density in each well of the plate has been recognized as one of the most important factors on the dynamic growth of cells. To enhance the stability of the developed method, the number of CHO-K1 cell seed in each well of the E-plate was optimized by adding different cell number/well ranging from 1000 to 35,000 cells/well. Cell adhesion and behavior were continuously monitored using CIB for 6 days. The dynamic growth curves of cells with different cell number/well were described in Fig. 2. As shown in Fig. 2, the CI values for all the wells are 0 before cell seeding into the wells. After cell titration, the CI value increases with the number of cells seeded into the well. When the number of CHO-K1 cell seeded in each well is too low, the lag phase of cell growth curve is prolonged obviously, which can not fulfill the requirement of quick cytotoxicity assay. On the contrary, the

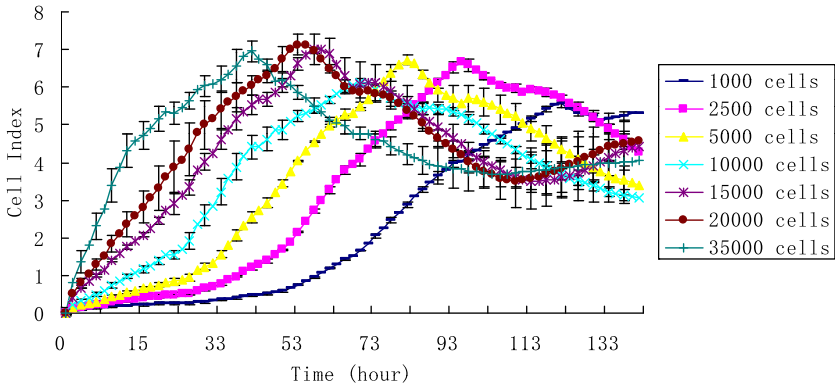

Fig. 2. Dynamic growth curves of CHO-K1 cells with different cell number/well monitored by the CIB method.

signals of the wells containing more than 5000 cells become unstable in the logarithmic phase (CV > 6%) because cells are suffering due to contact inhibition. Moreover, it is hard to differentiate the lag, logarithmic and stationary phase according to the growth curve of cells with high density. So, the optimal cell concentration was chosen based on their respective proliferation pattern. According to the experimental data, the impedance signal for the 5000 cells per well stabilizes (CV < 2%) and remains stable during the 4 days of testing, which suggesting the possibility to perform the cytotoxicity analysis. Therefore, 5000 cells per well was adopted as the initial cell number throughout the experiments.

The CIB could also provide valid evidences for cell quality control by monitoring the dynamic growth of CHO-K1 cells in real-time and differentiating its characteristic growth patterns. The cell adhesion and spreading kinetics, the time that the cells enter the log growth phase, and growth rate are characteristic of each cell type. Therefore, the dynamic growth curve of cell line described by CIB could be served as an ideal internal control and a method to standardize and validate stock cultures of cells over time. Compared with traditional methods with the light microscope, the CIB was not only a qualitative assessment of cell health, but also a quantitative one. Moreover, the dynamic growth curve of cell was helpful to pinpoint the optimal time points for compound treatment. In this study, the measurements of the dynamic growth curves of CHO-K1 cells were routinely performed to provide important information about cell quality and actual cell status before treatment.

3.3. Optimization of DMSO concentration in assay medium

DMSO has been widely used to dissolve the tobacco smoke condensate for in vitro cytotoxicity assays due to its moderate toxicity to test organisms and its excellent solvent properties for both polar and nonpolar compounds (Martino et al., 2006). In order to characterize the potential impact of this solvent, we assessed the effect of DMSO itself on the cytotoxicity. Fig. 3A demonstrates the cellular status changes induced by different concentration of DMSO using CIB. According to Fig. 3A, the CI values of all test wells almost exhibit the same profile at the initial stage before DMSO addition because of the same cells number. And then the DMSO induces a fast drop in the cell surviving rate during first 3 h of exposure. Afterwards, the cell surviving rates increases steadily. Fig. 3B illustrates the cytotoxic responses of DMSO with different concentration over a 24 h exposure period by both CIB and neutral red uptake. For CIB method, the cell viability was determined as the CI of the treated cells relative to the CI of the control after 24 h exposure. For NRU assay, the cell viability was determined as the neutral red uptake of the treated cells relative to that of the control after 24 h exposure. As depict in Fig. 3B, when the concentration of DMSO increases from 0.1% to 2.0%, the mean (SD) data of cell viability by the CIB method are 99(3)%, 96(3)%, 91(3)%, 85(3)%, 79(5)%, 77(5)%, 64(7)%, respectively. A significant reduction ($p < 0.05$) is observed at DMSO concentrations as low as 0.5% by CIB when compared with 0% DMSO control. However, when the concentration of DMSO increases from 0.1% to 2.0%, the mean (SD) data of cell viability by NRU method are 102(11)%, 103(8)%, 104(9)%, 102(4)%, 98(8)%, 90(11)%, 80(12)%, respectively. The DMSO-induced cytotoxicity was evident only at concentrations of 2.0% by NRU method when compared with 0% DMSO control ($p < 0.05$). So, the CIB-based method was proved to be more sensitive in detecting cytotoxic

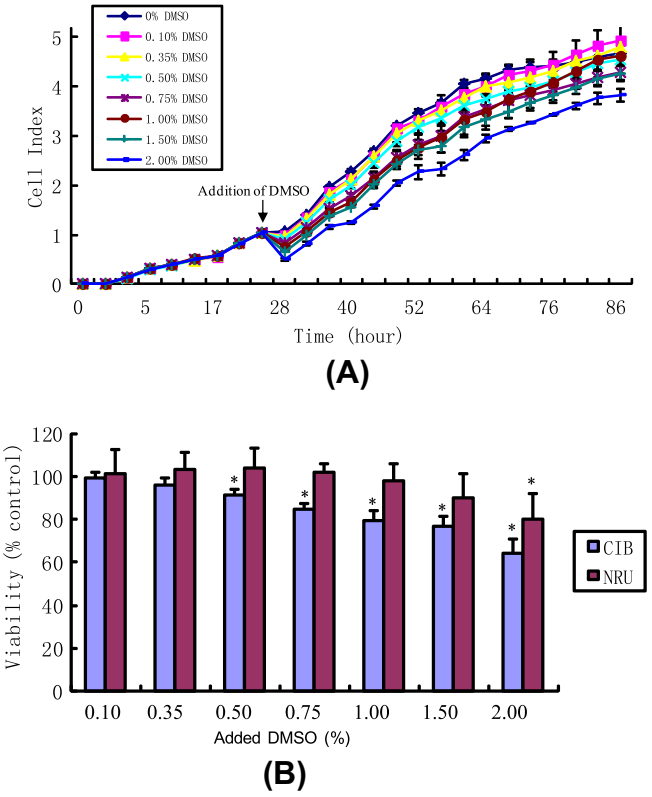

Fig. 3. The effect of varying the level of DMSO added to CHO-K1 cells on cytotoxicity by the NRU and the CIB method, respectively. (A) The dynamic cytotoxic response to different concentration of DMSO by the CIB method. (B) The viability of CHO-K1 cells after exposure to different levels of DMSO for 24 h by both the NRU and the CIB method. *Significantly different ($p < 0.05$) when compared with 0% DMSO control.

events compared with the neutral red uptake assay. A possible explanation for this observation is that the NRU assay only detects cell viability by determining the accumulation of the neutral red dye in the lysosomes of viable, uninjured cells, while the CIB considers cell morphology, cell adhesion to the well, and cell death or proliferation, making it more dynamic and sensitive than the NRU method in detecting cytotoxic events. In order to minimize the DMSO-induced toxicity, the final concentration of DMSO should never exceed 0.5% of the final volume in this CIB-based cytotoxicity assay.

3.4. Cytotoxicity assay by CIB

To obtain optimal compound concentration in evaluating the cytotoxicity of CSCs, serial concentrations of CSCs of cigarette sample A were added into respective designed wells containing 5000 CHO-K1 cells and the dynamic cell responses to different concentration of CSCs were continuously monitored using CIB. Controls with DMSO-only wells indicated normal cell growth in the electronic microwells, providing real-time control of cell status (growth and attachment to the microelectrodes). Fig. 4A represents the Nyquist plots obtained through the CIB electrode to characterize the impedance of gold interdigitated microelectrodes, a subconfluent layer of CHO-K1 cells and cell status change after exposure to CSCs. In Fig. 4A, the curve a shows the spectra of the empty electrode in culture medium without cells addition, the curve b corresponds to the spectra of the cell after 24 h in culture and the curve c represents the cell status after exposure to 150 μg/mL CSCs for 24 h. In the Nyquist plot the diameter of spectra increases depending on the cell spreading, cell growth and cell proliferation. After exposure to 150 μg/mL CSCs for 24 h, the diameter of spectra decreases because of the cytotoxicity effect of CSCs. Fig. 4B depicts the dynamic changes of CI values of all test wells after cell addition and exposure to CSCs with different concentration. As shown in Fig. 4B, the CI values of all test wells almost exhibit the same profile with the control cells in DMSO-only wells at the beginning of CSCs exposure, which indicating that the CSCs have no fast toxic effect on target cells in first 3–4 h. After 5 h exposure, all the concentrations of CSCs induced a gradually decline in the cell surviving rate in a dose-dependent manner. With higher concentrations of CSCs, CI could be reduced to zero which meaning no viable cells adhere to the microelectrodes. With lower concentrations of CSCs, the cells were able to continue growing

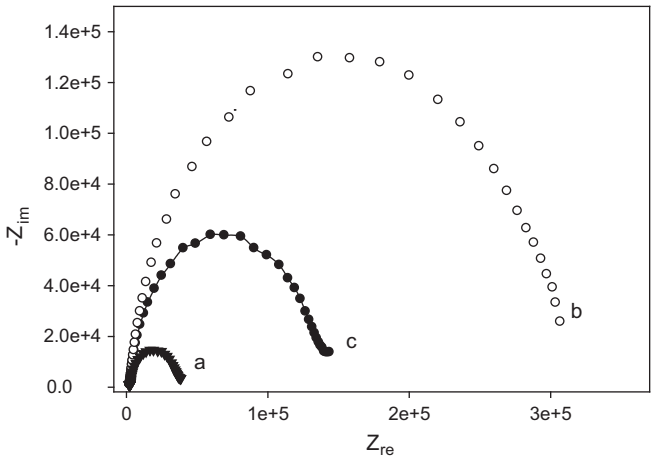

Fig. 4A. The Nyquist plots obtained through the CIB electrode. The curve a shows the spectra of the empty electrode in culture medium without cells addition, the curve b corresponds to the spectra of the cell after 24 h in culture and the curve c represents the cell status after exposure to 150 μg/mL CSCs for 24 h.

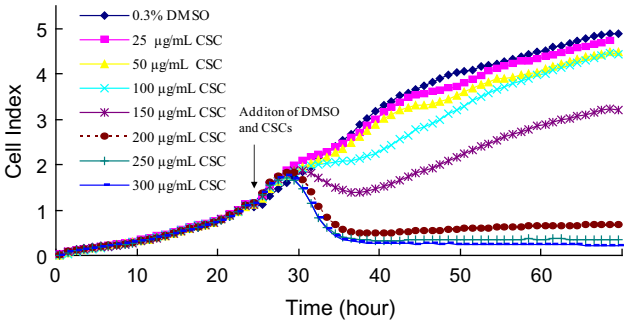

Fig. 4B. The dynamic cytotoxic response to different doses of CSCs using the CIB method.

with a slope similar to that of the control cells in DMSO-only wells during their log phase at approximately 15 h after addition of CSCs, representing the cell recovery responses to toxic agents. These curves gave important information about the cell growth and cellular status changes upon CSCs exposure in real time.

3.5. Time dependent IC_{50}

The IC_{50} value illustrates the effectiveness of a compound in inhibiting biological or biochemical function by quantitative measure the concentration of compound that causes 50% reduction in cell activity. Fig. 5A displays the cellular responses as a function of the log concentration of the CSCs (sample A) after 24-h exposure to the condensate. The IC_{50} value of sample A after 24-h CSCs exposure was obtained according to the dose–response CI curve. According to the dynamic cell responses to serial doses of CSCs shown in Figs. 4A and 4B, the CIB-based method allowed the calculation of a corresponding IC_{50} value at each time point. This time dependent IC_{50} curve could be exploited to identify the mechanisms for CSCs and demonstrate how fast the condensate effect is and if there are any other effects during the measurement. As shown in Fig. 5B, the cytotoxicity of CSCs to CHO-K1 can be detected at 4 h after condensates addition reaching an IC_{50} of 277.73 μg/mL. Thereafter, the IC_{50} value decreases gradually during the period of exposure. Finally, the IC_{50} value reaches a stable value and remains almost the same for exposure time longer than 10 h. The results depict that the cytotoxicity of CSCs enhanced with increasing exposure time and the optimal time point for calculating IC_{50} value was no less than 10 h exposure. The time dependent IC_{50} curve offered important advantages over the end-point measurement by supplying dynamic toxicity information and identifying the range of exposure time interesting to investigate.

3.6. Correlation between CIB and NRU assays

The NRU assay has been recommended by a Cooperation Center for Scientific Research Relative to Tobacco (CORESTA) In vitro Toxicology Task force as an in vitro assay to detect the cytotoxicity of cigarette smoke (Lou et al., 2009). In order to investigate the feasibility of the developed technique to be implemented for routine cytotoxicity testing, the IC_{50} values of the five commercial cigarettes were determined by both the CIB-based method and the NRU assay. These IC_{50} list in Table 2 are mean value of IC_{50} from six individual experiments (six replicates in each test) and the results indicated that the as-developed biosensor offered analytical results in good consistency with NRU assay for all the test samples (linear correlation coefficient of $R^2 = 0.9878$). The CV values between six individual tests by CIB were smaller than that of NRU assays for all the five cigarette brands owing to the desirable features of CIB in cell quality control, no cell labeling, and optimal time

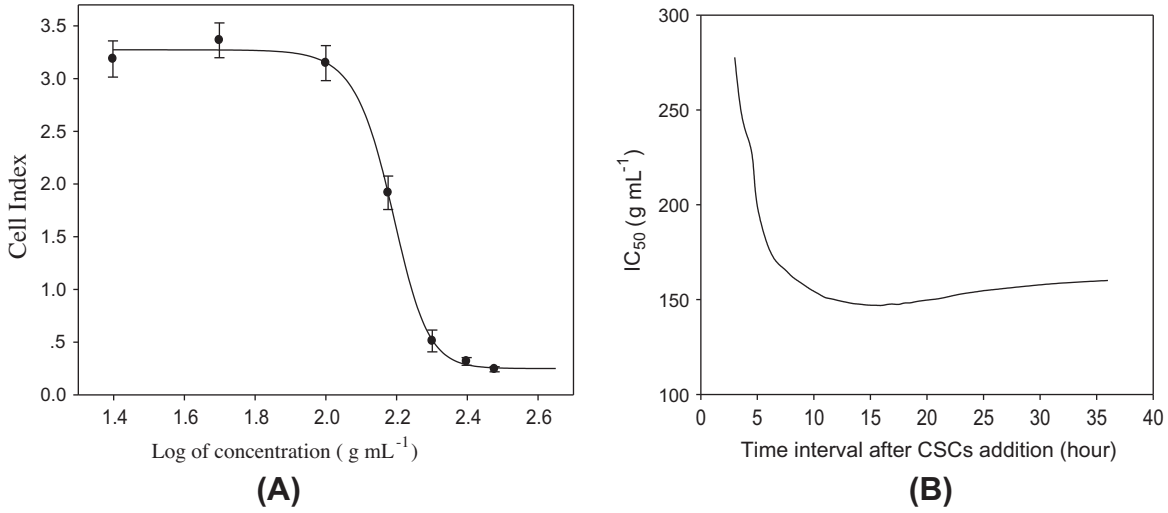

Fig. 5. (A) Effect of CSCs during 24 h exposure on the viability of CHO-K1 cells was measured based on the dose–response curves of the cell index by the CIB method. (B) Dynamic cytotoxicity information from relationship between IC_{50} and time for the CSCs.

Table 2
Comparsion of IC_{50} values of CSCs after 24-h exposure obtained by CIB and NRU assays.

Cigarette samples	CIB IC_{50} (μg/mL) [mean ± SD (n = 6)]	CV	NRU assay IC_{50} (μg/mL) [mean ± SD (n = 6)]	CV
A	154.440 ± 6.641	0.043	157.875 ± 11.841	0.075
B	150.250 ± 8.114	0.054	150.425 ± 11.131	0.074
C	110.630 ± 7.523	0.068	120.273 ± 12.388	0.103
D	99.183 ± 6.050	0.061	103.489 ± 11.384	0.110
E	91.016 ± 5.188	0.057	90.746 ± 7.804	0.086

point for compound treatment and endpoint assays. Moreover, only CIB supplies dynamic cytotoxicity information by monitoring cell status in real-time. The results indicate that the CIB-based method could be used as a replacement for NRU assay in characterizing the toxicity of different cigarette products.

4. Conclusion

The present study proposed a CIB-based method for assessing cytotoxicity of CSCs by real-time illustrating the actual kinetic cellular response including cell spreading, cell growth, morphological changes, and cell viability. In contrast with traditional endpoint methods, particular emphasis was placed on comprehensive representation of the cellular responses for the entire duration of the experiment, which supply a way to control cell quality and ensure optimal timing of manipulations or treatments. The cell number per well, the concentration of DMSO in assay medium, period of the exposure, and smoke condensate concentrations were easily optimized through the real-time dynamic curves of cellular response. Moreover, the time dependent IC_{50} curve obtained by the CIB-based method supplied important information about how fast the CSCs effect is and the cell recovery responses to CSCs during the measurement time. Additionally, the reproducibility and accuracy of cytotoxicity assessment by the CIB were also improved in contrast with the NRU method, which ensures the high sensitivity for cytotoxicity ranking of different cigarette products. Therefore, the proposed cellular impedance biosensor could be a promising technique for routinely cytotoxicity assays.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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