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# Impedance studies of bio-behavior and chemosensitivity of cancer cells by micro-electrode arrays

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ABSTRACT

Impedimetric analysis on adherently growing cells by micro-electrodes provides information related to cell number, cell adhesion and cellular morphology. In this study, cell-based biosensor with micro-electrode arrays (MEAs) was used to monitor the culture behavior of mammalian cancer cells and evaluate the chemosensitivity of anti-cancer drugs using electrochemical impedance spectroscopy. The platinum electrode arrays were fabricated by semiconductor technology to a $10 \times 10$ pattern, with diameter of 80 μm of each electrode. The human oesophageal cancer cell lines (KYSE 30) were cultured on the surface of the electrodes with the pre-coated fibronectin, the connecting protein for tumor cells metastasis and adhesion in extracellular matrix. Morphology changes during cells adhesion, spreading, and proliferation can be detected by impedimetric analysis in a real time and non-invasive way. Cisplatin was added to cells for potential drug screening applications. The experimental results show that this well-known anti-cancer drug has characteristic chemosensitivity effects on KYSE 30 cells which can be detected by MEA. Thus, this cell-based chip provides a useful analytical method for cancer research.



## 1. Introduction

In recent years, cancer is rapidly becoming the number one killer in many countries. And, chemotherapy (anti-cancer drugs) is still one of most important treatment methods in clinic. In pre-clinical testing studies, there is a great demand to develop more rapid and simple techniques for studying cancerous cells, especially for understanding their interactions with drugs and toxins. Conventional methods which are currently used in cancer cell biology mainly include fluorescent imaging, radioactive detection, and even animal experiments (Mosmann, 1983; Liu et al., 1997; Jepras et al., 1995). These techniques often need extensive experimental process and stringent laboratory conditions, besides instrumentation and cost. In addition, they are not able to provide continuous monitoring of a sample, and in consequence the information achieved may not reflect the real changes of cell activities at a specific time to a specific agent. In this case, there is a need to develop minimally invasive, reliable, inexpensive, and easy to use instrumentation for studying real-time biological events *in vitro* (Karasinski et al., 2005).

In the last decades, cell-based biosensors are the most studied novel techniques for cell monitoring methods due to their simplicity, sensitivity, and low cost (Bousse, 1996; Pancrazio et al., 1999). Using whole cells as the bio-recognition elements, those sensors can detect agents functionally with physiological changes to cells (Wang et al., 2005; Rasooly and Jacobson, 2006; Yu et al., 2007). One kind of them is sensor using electrochemical impedance spectroscopy, which is first reported as electric cell-substrate impedance sensing (ECIS) technique for cell proliferation, morphology, and motility monitoring (Giaever and Keese, 1984, 1993; Keese et al., 2004). The basic idea of the measurement is that when mammalian cells attach and spread on the surface of a gold electrode, the cells essentially hinder unrestricted current flow from the electrode into the bulk electrolyte and thereby increase the overall electrode impedance. Currently, ECIS as well as other commercially available systems have been developed and applied as one of the most interesting label-free technology for studying cell adhesion on a surface and give real-time and kinetic information of cell behaviors. Recently, those impedance biosensors have been used for assessment of cytotoxicity (Xiao and Luong, 2005; Ceriotti et al., 2007),

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pharmacological apoptosis (Arndt et al., 2004; Yin et al., 2007), and even drug affections related with special disease (Yang et al., 2007).

In the case of cancer research, cell adhesion into extracellular matrix is the precondition to tumor metastasis, and then tumor clone is formed with unbounded cell proliferation. The current adhesion assay is an *in vitro* method which is used to determine the rate or strength of adhesion for different cell types to extracellular matrix proteins by fluorescent labeling (Murphy-Ullrich, 2001; Okeqawa et al., 2004). At the same time, cellular morphology is also one of most important parameters in cancer biology at all times. Especially, most of anti-cancer agents currently employed that target the cytoskeleton, do so through interactions with the microfilaments (Rao and Li, 2004). The cytoskeleton consists of a complex network of filamentous proteins which are involved in regulation of cell morphology and adhesion. Moreover, the concept that tumor cells when exposed to anti-cancer drugs usually die from apoptosis has become a widely held tenet of modern cancer treatment (Hengartner, 2000; Buolamwini and Adjei, 2003).

Through above analysis, we can find that cellular adhesion, proliferation, and morphology change induced by apoptosis can be monitored with impedance sensors. Thus, this sensing technology could be used for multiple purposes in cancer cell biology and therapeutics. Maybe, they could complement conventional cell culture methods for studying biological processes which are involved in cell proliferation or as screening devices for drug testing. In recent years, some groups have reported micro-biosensor chips using impedimetric analysis for cancer clinical testing (Chen et al., 2008; Jang and Wang, 2007; Cho and Thielecke, 2007; Linderholm et al., 2007; Klo et al., 2008; Campbell et al., 2007; Han et al., 2007). For example, human ovarian cancer cells and human hepatocarcinoma cell lines were cultured on the surface of quartz crystal or indium tin oxide electrodes for cell's adhesion and cytotoxicity detection by impedance analysis (Li et al., 2005; Guo et al., 2006). Those cells were just used as a kind of cell source, and their tumorous characteristics and chemosensitivities were never thoroughly investigated.

In this study, the human oesophageal cancer cell lines (KYSE 30) are cultured on the surface of the micro-electrodes coated with fibronectin to monitor cancer cells' adhesion, proliferation, and morphology by impedance monitoring. Then, the well-known anti-cancer drug of cisplatin was added to prove if this sensor system is a useful *in vitro* method for chemosensitivity evaluation.

## 2. Experiments

### 2.1. Fabrication of the electrode arrays

The fabrication process of the electrode arrays has been described in our previous paper (Yang et al., 2005). Briefly, a silicon wafer (2 in. diameter) with a PECVD grown silicon nitride insulating layer (2000-Å thick) was used as the starting materials. First, a platinum layer (1000-Å thick) was deposited onto the substrate using electron beam vapor deposition. Then, the platinum layer was patterned using photolithography. The electrode array pattern was formed by wet etching in 3–5% HF solution. Finally, a polyimide film was applied to the substrate by spin-coating and patterned by reactive ion etching. A micrograph of the fabricated micro-electrode array is shown in Fig. 1 with a $10 \times 10$ array pattern. The electrode diameter is 80 μm with a 200 μm center to center spacing. Two platinum plated leads (6-μm, thick) from each electrode terminated at two separate electrode pads. The dimensions of the electrode pads were 5 cm × 5 cm.

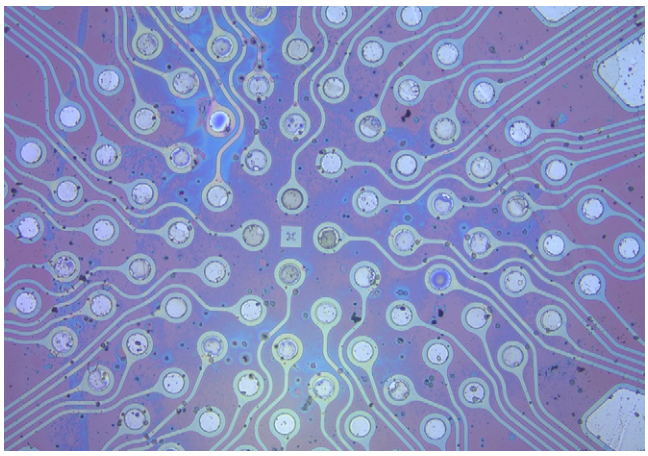

**Fig. 1.** Photo of the micro-electrode arrays ($10 \times 10$ with diameter of 80 μm).

### 2.2. Device and cell culture

A 5 cm × 5 cm × 1 cm micro-chamber was formed by PDMS (polydimethylsiloxane) on the micro-electrode chip for cell culture. The cell line employed in our project is the human oesophageal cancer cell lines (KYSE30) obtained from American Type of Culture Collection. Cells were maintained routinely in minimum essential medium (JRH BioSciences) supplemented with 5% fetal bovine serum (Hyclone) together with penicillin and streptomycin (Invitrogen). They were cultured on a 35 mm diameter tissue culture dish (Nunc GmbH & Co. KG, Germany) in a humidified incubator at 37 °C with 5% $CO_2$/95% air. About 20 ml of 1–2 million cells/ml suspension was plated onto sensor coated with fibronectin (10 mg/ml) prior to cell seeding. The density and morphology of cells on substrate surface were investigated with a microscope (Nikon Eclipse FN1, Japan).

### 2.3. Impedance detecting

The impedance detecting setup was shown in Fig. 2. During experiments, sensor chip with cultured cells was inserted into a chip cartridge, with 40 independent electromechanical relays. Each relay has a normally closed, normally open, and common terminal. For the array of electrodes, each sensing electrode had a solderable pad at the edge of the chip. Wires connected to common terminals were soldered to these pads. Then, the cartridge was put into incubator with wires contacted to detecting machine. Culture medium and drug solution containing anti-cancer drugs were injected alternatively by a peristaltic pump into the micro-chamber through a degasser and a selection valve. All the measurements were performed at 37 ± 0.2 °C in the incubator.

Impedance spectra was performed using the impedance analyzer VersaSTAT3 (Princeton Applied Research, USA) controlled by a personal computer. The impedance between voltage and current were recorded within a frequency range of 1 Hz to 1 MHz. A pure sinusoidal AC voltage of 10 mV amplitude (peak-to-peak) was applied and 100 data points per frequency decade chosen to be equidistant on the logarithmic scale were recorded, which needs about 7 min to complete a frequency domain scan. Then, impedance of the electrodes in the cell culture medium was measured at a fixed frequency (10 kHz) every minute during the impedance sweep interval to obtain real time recording.

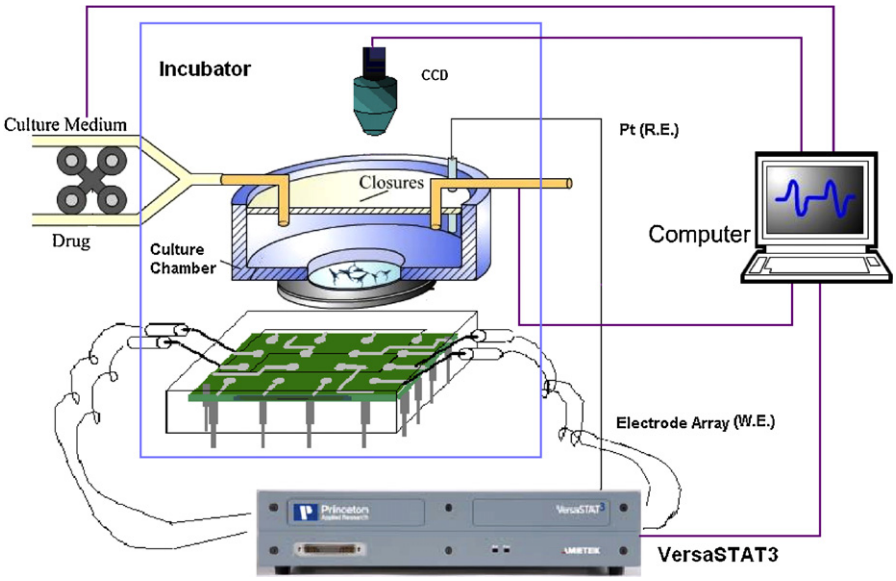


**Fig. 2.** The schematic diagram of impedance detecting system (electrodes and connecting lines are only representatives, not the actual number).

### 2.4. Pharmacology

To validate the applicability of this cancer cell-based biosensor in pharmacological bioassays, a well-established anti-caner drug of cisplatin (Sigma–Aldrich Co., USA), was added for potential chemosensitivity screening applications. Various concentrations (10, 25, and 50 μM) of the pharmacological agents were prepared in normal culture solution. Basal parameters were recorded after cells cultured on the chips about 24 h, followed by complete replacement of the culture medium with drugs in a certain concentration. Recordings were made in the presence of drugs for 16 h.

### 2.5. Fluorescence imaging

Cell morphology is mainly maintained by cytoskeleton which is composed of microtubule, microfilament and intermediate fiber. Changes of the microfilament cytoskeleton also could directly manifest adhesion and metastasis characteristics of cancer cells. Thus, fluorescent imaging was performed using the fluorescent conjugates of phalloidin, which are generally used to label actin filaments to see cancer cell morphology change and propidium iodide to see nucleic acid (Sigma–Aldrich Co., USA). In our study, cells were fixed for 5 min in 3.7% formaldehyde, and permeabilized with 0.1% Triton X-100, and followed by staining with a 50 ug/ml fluorescent phalloidin conjugate or propidium iodide for 40 min at room temperature. Then, stained cells were observed using the fluorescent microscope (Nikon ECLIPSE 80i, Japan). Excitation and emission wavelengths were 499 and 599 nm, respectively.

## 3. Results and discussion

### 3.1. Cellular adhesion on the electrodes coated with fibronectin

Fibronectin is a major extracellular matrix component of cancer-associated biological stroma. Because interactions mediated by fibronectin are implicated to modulate cancer cell's invasion to normal cells and tissues through matrix. Here, we examined the cell adhesion on two types of micro-electrodes: one was pre-coated with fibronectin and the other was a non-treated surface. Fig. 3A shows that the KYSE30 cells adhered very well on those fibronectin-treated electrode surfaces after 24 h. The cell density was about

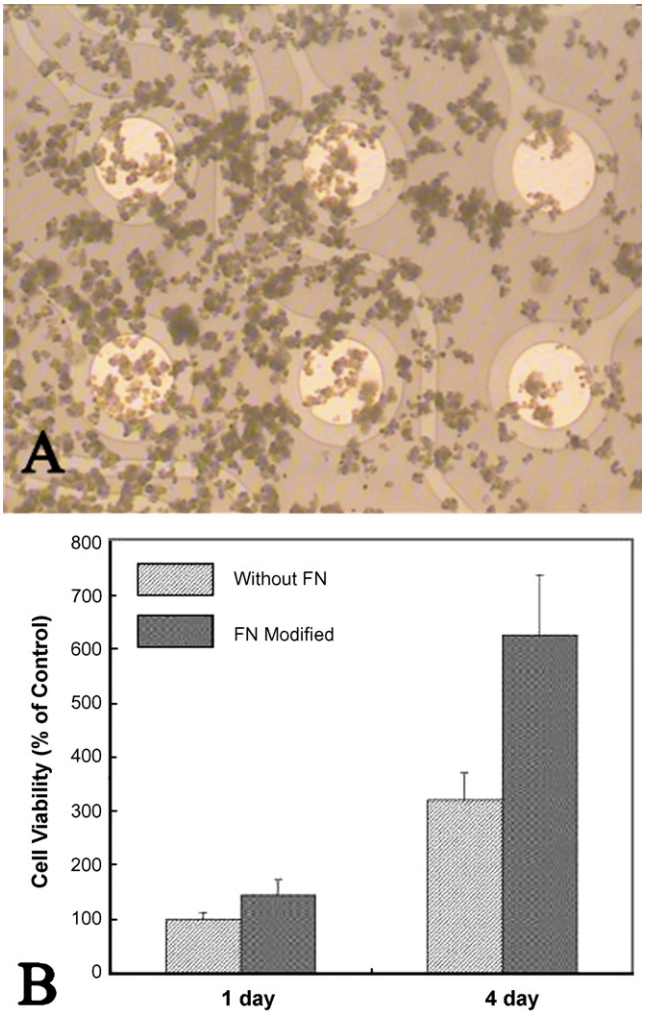


**Fig. 3.** KYSE30 cells cultured on the electrode surface. (A) Cells adhered on fibronectin (FN)-modified electrode after 24 h observed by the Nikon FN-1 microscope; (B) comparison of the average absorbance values of cells on different electrode surfaces by MTT-based assay.

$2.8 \times 10^4$ cells/cm$^2$ on the non-treated surface, whereas it was $5.1 \times 10^4$ cells/cm$^2$ on the fibronectin-modified surface. Fig. 3B shows the result of the MTT assay. The average absorbance values were expressed as a percentage of the control, unmodified electrode surface (at day 1). KYSE30 cells cultured on fibronectin-modified sensor surface exhibits significantly higher cell viability compared with that of cells on the non-treated surface at 4 days of culture.

### 3.2. Impedance detection

The cell-based biosensor using micro-electrodes was used to perform preliminary tests of the impedance technique with cancer cells cultured on the chip. After impedance spectra were monitored within a frequency range of 1 Hz to 1 MHz to get frequency-dependent recordings, real time measurements were performed at a fixed frequency to get time-dependent recordings.

Fig. 4A shows the typical frequency-dependent impedance spectrum before and after KYSE30 cell lines were cultured on the electrodes, with a fitting equivalent circuit representing the impedance spectrum of our system (Yang et al., 2007; Arndt et al., 2004). The impedance spectrum is evaluated by fitting the appropriate equivalent circuit parameters to the experimental data (Tlili et al., 2003; Yang et al., 2007). As demonstrated before, each element of the circuit contributes predominantly to different frequency regimes, which allows separation of the effects of the different components of the electrical interface. The resistor $R_S$ is mainly due to the conductivity of the bulk solution and the wire connection. The $Z_{CPE}$ represents the dielectric properties of the electrode/electrolyte and surface morphological information. $R$ and $C$ are the resistance and the capacitance of the layer of the absorbed proteins. $R_c$ and $C_c$ are elements related to the cell morphology change. Values of $R_S$, $Z_{CPE}$, $R$ and $C$ remain constant in the equivalent circuit while values of $R_c$ and $C_c$ components vary with cells changes. Our results show that this simple model could properly fit the measured data over the whole frequency rage.

Comparing impedance spectrum before and after KYSE 30 cell lines cultured, the difference in the total spectrum indicated that impedance reading at 100 Hz to 100 KHz passes a maximum value. So we selected 10 KHz as the fixed frequency to obtain real time process of cells adhesion. Fig. 4B shows a typical result for an adhesion process. In this particular case, cells (150,000 cells/ml) were adsorbed to fibronectin pre-treated or non-treated surfaces. The impedance prior to cell addition is taken as the reference state. When cells attached on the electrodes, typical impedance magnitude increased greatly upon cell adhesion which was in the range of a few 100 Ω. The time needed to reach the stationary state was about 1 h. Then, the impedance increased slowly and steadily in the next 16 h. This adhesion process was mainly due to cells adhesion, spreading and proliferation, comparing with cells density and morphology investigated by CCD camera (Li et al., 2005). However, the increasing rate and range to this increasing stationary state displayed reproducible variability, depending on the number of cells and the cell–surface interactions that were mainly coursed by fibronectin in this study.

Researchers of cancer metastasis have gained insight into the mechanisms by which metastatic cells arise from primary tumors tend to metastasize to specific organs (Fidler, 2003). An important issue is that metastasis depends on cross-talk between selected cancer cells (the 'seeds') and specific organ microenvironments (the 'soil'), which depends on tumor cells interactions with the homeostatic factors that promote tumor cell growth, survival, angiogenesis, invasion and metastasis. Therefore, this impedance detection for cancer cells seeding to bio-molecular (i.e. fibronectin)-modified surface will be a very good *in vitro* model for cancer cell bio-behavior studies.

### 3.3. Drug's chemosensitivity analysis

Reliable assessment of cell death is a very important issue in developing effective and safe anti-cancer drug therapies. Many current used drugs kill cancer cells through apoptosis. A cost-effective platform to screen drugs based on cellular death and apoptosis will improve the development process greatly. Impedance detection has been shown to monitor apoptosis-induced changes of cell shape in real-time, differing from detection tools such as fluorescence microscopy and flow cytometry (for apoptosis cells) with caspase-3 or DNA fragmentation assay kits (Asphahani and Zhang, 2007; Arndt et al., 2004). In this study, we used chemotherapy drug of

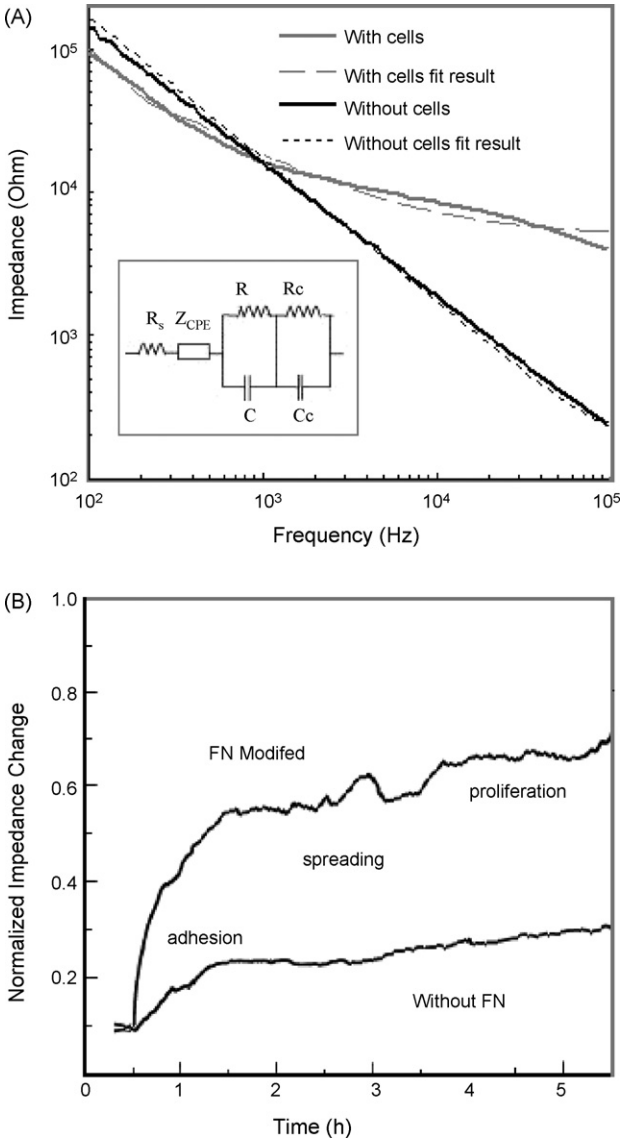


**Fig. 4.** One channel impedance detection of the micro-electrode array for cancer cells adhesion. (A) Measured impedance frequency spectrum without cells and with cells cultured on the electrode surfaces for 24 h. The impedance spectrum is evaluated by fitting the appropriate equivalent circuit parameters to the experimental data. (B) Time course of the impedance magnitude at a sampling frequency of 1 kHz for fibronectin (FN) modified and non-modified electrode surfaces.

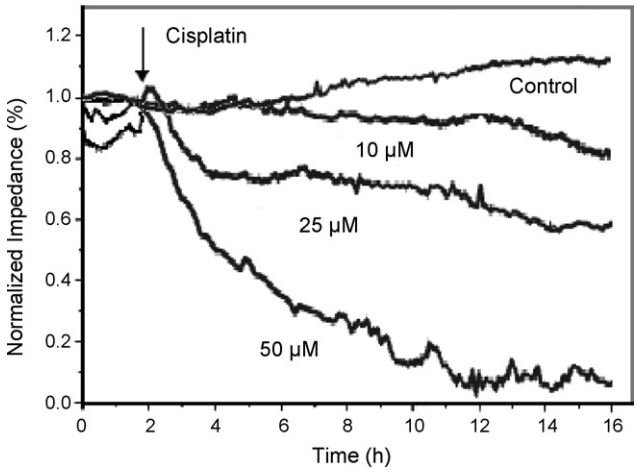


**Fig. 5.** Chemosensitivity analysis of cisplatin for KYSE30 cells. Cells were treated with cisplatin (10, 25, and 50 μM), and impedance was recorded for up to 16 h with culture media as control. This is a representative graph of one of electrode channels' impedance normalized to baseline. Arrow indicates time of cisplatin addition under the culture conditions.

cisplatin to prove the potential anti-cancer drug screening applications of our system.

The direct effects of cisplatin on the cultured KYSE30 cells were shown in Fig. 5. Stimulation with different concentrations of cisplatin (10, 25, and 50 μM) all caused decreases of impedance from baseline, which showed an obvious concentration-dependent effect. However, impedance changes with different concentrations showed different characteristics during the stimulation period. Both 25 and 50 μM cisplatin induced a slightly progressive increase of amplitude for about 20 min. Then, the impedance continued to decrease until a steady state was reached after 6 h. Finally, in the steady state of 12 h, impedance amplitude of 50 μM cisplatin no longer changed, which concluded that the cancer cells have been completely killed at that time. For the lower concentration of 10 μM cisplatin, the impedance amplitude did not show an obvious decrease until 5 h after the addition of cisplatin.

Because most of the anti-cancer agents currently employed that target the cytoskeleton, do so through interactions with the microtubules, conventional fluorescence imaging was explored to testify that the impedance amplitude decrease upon the addition of cisplatin is due to the cell morphology change. Fig. 6 shows the example imaging of KYSE30 cells stimulated by 25 μM cisplatin. As shown in Fig. 6A, typical cell spreading and cytoskeletal organization were observed in the absence of cisplatin. Cisplatin reduced the abundance of actin filaments (bright red in the image) over the course of 12 h, as shown in Fig. 6B. The loss of the filaments resulted in less cell–substrate contacts and the decreased cell covering area on the surface of the micro-electrodes. The fluorescent images with propidium iodide staining for nucleic acid were also shown (Fig. 6C and D). After 12 h of cisplatin addition, a series of characteristics including loss of cell volume, clumping of chromatin and nuclear fragmentation were observed (Fig. 6D). All of these morphological changes suggested that addition of cisplatin could lead to the obvious morphology change such as shrinkage of cell spreading area, loss of cell–substrate contacts, even round-up of cells, which in turn deceased the related impedance amplitude.

Cisplatin is an active medicine against many cancers, which crosslinks DNA in several different ways, and then interfering cell division by mitosis. DNA repair mechanisms are elicited by the damaged DNA. And then apoptosis is activated when repair proves impossible. All of these pharmacological changes are related with cell's morphology changes. In our study, when cisplatin was

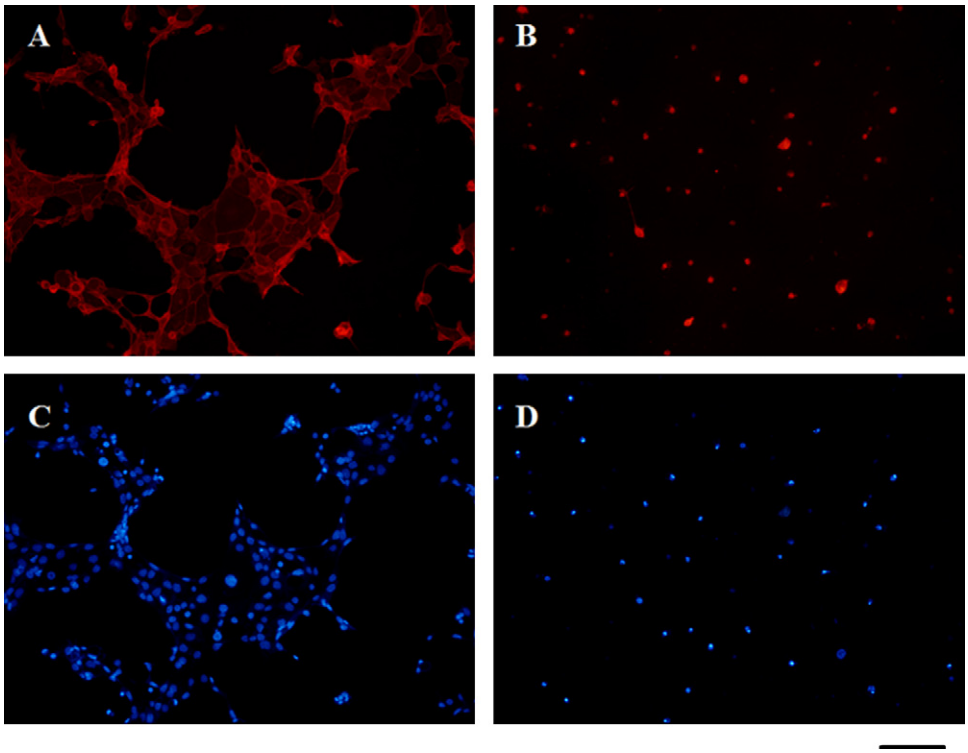


**Fig. 6.** Fluorescence imaging of KYSE30 cells cultured on the surface of the micro-electrodes. (A) Phalloidin imaging specific for microfilament after cells cultured 48 h; (B) phalloidin imaging after cells treated with cisplatin 12 h; (C) propidium iodide specific for nucleic acid imaging after cells cultured 48 h; (D) propidium iodide specific for nucleic acid imaging after cells treated with cisplatin 12 h. (Bar is 50 μM).

added to cells, the impedance even decreased with different characteristics. All of these suggested that different drugs (or even with different concentrations) have different mechanisms to cancer cells. Therefore, this impedance biosensor system can be used for pharmacokinetics studies, with merits of real time recording.

## 4. Conclusion

A cell chip with micro-electrode arrays was used to monitor the culture behavior of mammalian cancer cells and evaluate the chemosensitivity of anti-cancer drug using electrochemical impedance spectroscopy. Morphology changes of cells adhesion, spreading, and proliferation can be detected by impedimetric analysis. Anti-caner drug of cisplatin was added to cells for potential drug screening applications. The experimental results showed that this well-known anti-cancer drug has chemosensitivity effects which can be detected by microelectrodes.

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