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A disposable impedance sensor for electrochemical study and monitoring of adhesion and proliferation of K562 leukaemia cells

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Abstract

A polyaniline-modified screen-printed carbon electrode (PANI/SPCE) was prepared by electropolymerization for the construction of a novel disposable cell impedance sensor. The conductive polymer improved greatly the electron transfer of SPCE and was very effective for cell immobilization. The adhesion of cells increased the electron transfer resistance (R_{et}) of redox probe on the PANI/SPCE surface, producing an impedance sensor for K562 leukaemia cells with a semilogarithm linear range from 10^4 to 10^7 cells ml^{-1} and a limit of detection of 8.32×10^3 cells ml^{-1} at 10σ. The proliferation of cells on the conductive polymer increased the R_{et}, leading to a novel way to monitor the growth process of cells on the PANI/SPCE. The electrochemical monitoring indicated K562 leukaemia cells cultured *in vitro* on the PANI surface were viable for 60 h, consistent with the analysis from microscopic imaging and MTT assay. This method for monitoring the surface proliferation and detecting the number of viable cells was simple, low-cost and disposable, thus providing a convenient avenue for electrochemical study of cell immobilization, adhesion, proliferation and apoptosis.

Keywords: Electrochemical impedance spectroscopy; Impedance sensor; Cell proliferation; K562 leukaemia cells; Polyaniline; Screen-printed carbon electrode

1. Introduction

Cellular adhesion and proliferation have attracted considerable attention due to their critical role in unraveling the life process. Electrochemical techniques have become the very powerful tools for studying living cells. In these techniques anchoring cells on a proper surface invariably constitutes a key step [1]. As a result, a growing interest has focused on the search of new culture substrates [2,3]. Polyaniline (PANI), one of the well-studied conducting polymers, possesses intriguing electrical, electrochemical, and optical properties [4] and has been extensively utilized for preparation of electrochemical biosensors [5,6], cellular adhesion [7] and tissue engineering [8]. The PANI electropolymerized on an electrode can present extensive amount of amine groups, which are positively charged in aqueous cell culture medium, thus offering electrostatic anchoring points for cells with negative-charged surfaces. This work used conductive PANI to improve the electric behaviors and biocompatibility of screen-printed carbon electrode (SPCE) for the development of a disposable cell-based sensor.

SPCE has been used as a support to fabricate various biosensors or devices [9,10]. This technology allows the mass production of simple, reproducible yet inexpensive and mechanically robust strip solid electrodes. Other important features are related to the miniaturization of the corresponding device along with their ease of handling and manipulation in a disposable manner, which can be associated with designer PANI for fabrication of the cell sensor in this work. The proposed disposable sensor for cells was based on the change of electron transfer resistance (R_{et}) of redox probe on the PANI/SPCE surface upon immobilization of cells. The improvement of electric behaviors by the conductive PANI increased the sensitivity

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of the sensor. The biocompatibility of PANI made it possible to monitor the adhesion and proliferation of immobilized cells on SPCE surface.

Electrochemical impedance spectroscopy (EIS) has been used for development of cell sensors [11–13]. An impedance-based cell sensor for *Escherichia coli* O157:H7 has been prepared on an indium–tin oxide interdigitated array microelectrode [13]. In comparison with surface plasmon resonance and quartz crystal microbalance techniques, EIS technique is low-cost and relatively easy to use. Herein the prepared amine-based SPCE surface was demonstrated to be very effective for immobilization of tumor cells and provided a simple, low-cost and disposable avenue for cell impedance sensing and electrochemical study of cell adhesion, proliferation and apoptosis of living cells on interface.

2. Experimental

2.1. Materials and reagents

Aniline with analytical reagent grade was purchased from Shanghai Chemical Reagent Co, LTD. (China) and distilled before use. Phosphate buffer saline (PBS) (pH 7.4) containing 137 mmol l^{-1} NaCl, 2.7 mmol l^{-1} KCl, 87.2 mmol l^{-1} Na_2HPO_4 and 14.1 mmol l^{-1} KH_2PO_4 was used as the electrolyte. Potassium ferrocyanide and potassium ferricyanide were purchased from Shanghai Chemical Reagent Co. LTD. (China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO). Sodium dodecyl sulfate was obtained from Huakang Science & Technology Limited (China). The solutions were prepared with deionized water of 18 MΩ purified from a Milli-Q purification system.

2.2. Cell line and cell culture

K562 cell line was kindly provided by the Affiliated Zhongda Hospital, Southeast University, Nanjing, China. K562 cells were cultured in a flask in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 μg ml^{-1}) and streptomycin (100 μg ml^{-1}) at 37 °C in a humidified atmosphere containing 5% CO_2. After 72 h, the cells were collected and separated from the medium by centrifugation at 1000 g for 10 min, and then washed twice with a sterile pH 7.4 PBS [2]. The sediment was re-suspended in the PBS to obtain a homogeneous cell suspension with the final concentration of 2.0×10^6 cells ml^{-1}. Cell number was determined using a Petroff–Hausser cell counter (USA).

2.3. Preparation and modification of SPCE and cell immobilization

The combining three-electrode system was printed on a polyvinyl chloride (PVC) membrane (0.025 mm thickness) (Fig. 1) using a SH/300F screen-printer (Ever Bright Printing Machine Factory Ltd., China) as described in a previous work [10]. The diameter of carbon disc was 4 mm (0.12 cm^2). The prepared SPCE was firstly subjected to cyclic scanning in 0.2 mol l^{-1} H_2SO_4 in a potential range of −0.2 to +1.1 V until reproducible voltammograms were obtained. Polymerization of aniline was then achieved in a potentiodynamic mode in 1.2 mol l^{-1} HCl solution containing 0.2 mol l^{-1} aniline with cyclic scanning between −0.2 and +1.1 V at 100 mV s^{-1} for 10 cycles under nitrogen atmosphere. Following a rinse with deionized water, K562 cell suspensions with different cell numbers (10 μl) were dropped onto the surfaces of PANI/SPCE. After water was evaporated at 37 °C, the K562 cells adhered PANI/SPCE (cells/PANI/SPCE) was washed thoroughly with deionized water and used for electrochemical measurements. The scheme of SPCE modification steps was shown in Fig. 1A.

2.4. Cell proliferation on PANI/SPCE and MTT assay

K562 cells (10 μl 1.0×10^5 cells ml^{-1}) were introduced onto the surfaces of a batch of PANI modified SPCEs and cultured in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10% FCS, penicillin (100 μg ml^{-1}) and streptomycin (100 μg ml^{-1}) at 37 °C in a humidified atmosphere containing 5% CO_2. After K562 cells were cultured on the PANI films for different periods, these cell-proliferated PANI/SPCEs were taken out of culture medium for EIS measurements, respectively. The photos of K562 cells proliferated on the PANI/SPCE were recorded on an optical microscope (DMLP, Leica) with a magnification of 500×.

For MTT assay, the K562 cells adhered on the PANI film with various culture times were collected to a well of 96-well plate in a volume of 200 μl medium. MTT (20 μl, 5 mg ml^{-1}) was then added to each well. After the plate was incubated for 4 h, sodium dodecyl sulfate (150 μl, 0.5 mol l^{-1}) was added to each well to solubilize formazan dye. After incubation for 1 h, the absorbance was measured on a Wallac Victor2 1420 Multilable Counter at 490 nm.

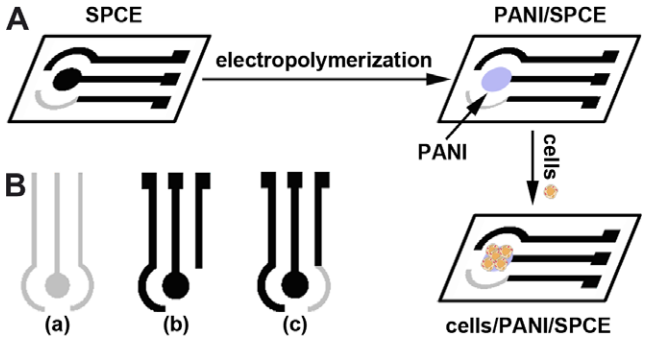

Fig. 1. Schemes for fabrication of the cell-based impedance sensor (A) and SPCE (B). (a) Sliver layer, (b) carbon layer and (c) SPCE product.

2.5. Apparatus and characterization

Cyclic voltammetric measurements were performed on a CHI 730 electrochemical analyzer (CH Instruments, Inc.). EIS measurements were carried out on a PGSTAT30/FRA2 system (Autolab, Netherlands) in 100 μl 0.01 mol l^{-1} pH 7.4 PBS containing 10 mmol l^{-1} $Fe(CN)_6^{3-/4-}$ and 0.1 mol l^{-1} KCl. The impedance spectra were recorded within the frequency range of 10^{-2}–10^6 Hz with 5 mV amplitude of the applied sine wave potential. All the EIS experiments were performed at 37 ± 0.5 °C. Attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) was carried out on a NEXUS 870 FTIR (Nicolet, USA) equipped with an omni sampler over 32 scans.

3. Results and discussion

3.1. Cyclic voltammetric behavior of PANI/SPCE

Electropolymerization of the monomer aniline was performed with potentiodynamic technique [14]. The electropolymerization conditions were chosen according to Mathebe's work [15]. Inset in Fig. 2A showed the cyclic voltammogram for electropolymerization of aniline on a SPCE in 1.2 mol l^{-1} HCl solution. The electrochemically synthesized PANI layer was electroactive in the studied potential region, exhibiting two pairs of stable and well-defined redox peaks (Fig. 2A). The couple of redox peaks labeled as A and B were attributed to intrinsic redox process of the transformation of PANI from the reduced leucoemeraldine state to the partly oxidized emeraldine state [15]. Another couple of peaks labeled as C and D were generally attributed to the redox reaction of *p*-benzoquinone [16]. All these redox peaks increased with the repeated potential scanning during polymerization process, indicative of polymer deposition at the SPCE surface, and confirming that the polymer was electronically conductive.

The formation mechanism of PANI has been widely studied [4,17]. It is believed that aniline is firstly oxidized to form aniline radical cation, which reacts with another aniline molecule to form a dimer cation. After deprotonation the formed dimer (*p*-aminodiphenyleneamine) undergoes further oxidation and coupling to form tetramers. The oxidation and coupling processes continue until a polymer is formed [18]. With the increasing scan rate, the peak currents and the peak-to-peak separations of two couples of peaks increased (Fig. 2B). The peak currents were proportional to $v^{1/2}$, indicating that the diffusion of electrons was taking place alone the polymer chain.

3.2. ATR-FTIR spectra of PANI/SPCE and K562 cells proliferated on the PANI/SPCE

The ATR-FTIR spectrum of the PANI/SPCE showed an absorption peak at 3210 cm^{-1} (Fig. 3A), which was ascribed to the N—H stretching vibration of PANI. The peaks at 3000, 1580, 1500 and 750 cm^{-1} were characteris-

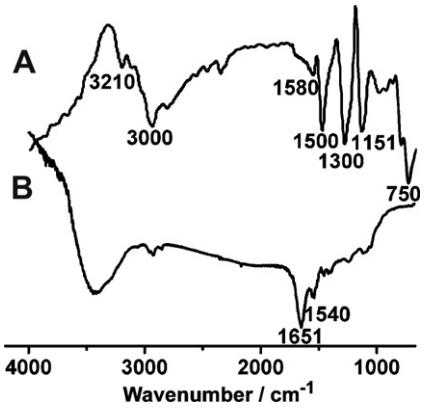

Fig. 3. ATR-FTIR spectra of PANI film formed on the SPCE (A) and K562 cells proliferated on the PANI/SPCE by introducing 10 μl 1.0×10^5 cells ml^{-1} K562 cells onto the surfaces of PANI modified SPCE and culturing at 37 °C for 60 h (B).

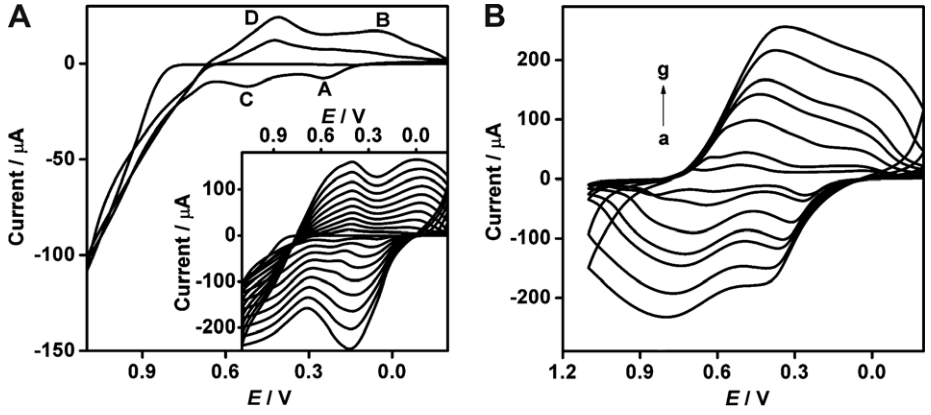

Fig. 2. Cyclic voltammograms of (A) the first two cycles recorded during the electropolymerization of aniline in 1.2 mol l^{-1} HCl containing 0.2 mol l^{-1} aniline at 100 mV s^{-1} with the initial potential of −0.2 V and (B) the PANI/SPCE in 1.2 mol l^{-1} HCl at 10 (a), 20 (b), 50 (c), 80 (d), 100 (e), 150 (f) and 200 (g) mV s^{-1}. Inset in (A): cyclic voltammograms recorded during the electropolymerization of aniline for 10 cycles.

tics of the various vibrations of the C—H and C—C bonds of the aromatic nuclei, and that at 1300 cm^{-1} was assigned to the stretching vibration of the C—N bond of the secondary aromatic amine [19]. After K562 cells were proliferated on the PANI film, these absorption peaks were unobservable due to the dense covering of the living cells, while two new absorption peaks occurred at 1651 and 1540 cm^{-1}, which were similar to the bands of proteins for amide I groups at 1610–1690 cm^{-1} corresponding to the C=O stretching vibration of peptide linkages and amide II groups around 1500–1600 cm^{-1} from a combination of N—H bending and C—N stretching [20]. The weak bands occurred in the region of 1200–1400 cm^{-1} were assigned to the wagging and twisting vibrations of the $-CH_2$ group in these proteins and were commonly referred to as the progression bands [21]. These infrared absorption bands verified the adhesion of K562 cells on the surface of the PANI film and the preservation of the native structure of proteins on cell membrane.

3.3. Cell proliferation study and MTT asssay

The morphology of the adhered K562 cells was studied using the optical microscopy after they were seeded on the PANI film. K562 cells were able to adhere to the PANI film after incubation for 10 h (Fig. 4A). With the increasing incubation time from 10 to 60 h, the proliferation of these cells on the film could be observed with an increase in K562 cell number. These proliferated cells showed an obvious spreading appearance, and the morphology of distinguishable filopodia, which were the evidence of cell adhesion to material surface and the indication of good viability of cells [22]. A longer incubation time caused the loss of normal cellular characteristics and viability, presenting an abnormal morphology on the film.

Generally, the absorbance of MTT assay is proportional to the number of viable cells. The results of MTT assay of the K562 cells samples obtained from the K562 cells-seeded PANI films with different incubation times were shown in

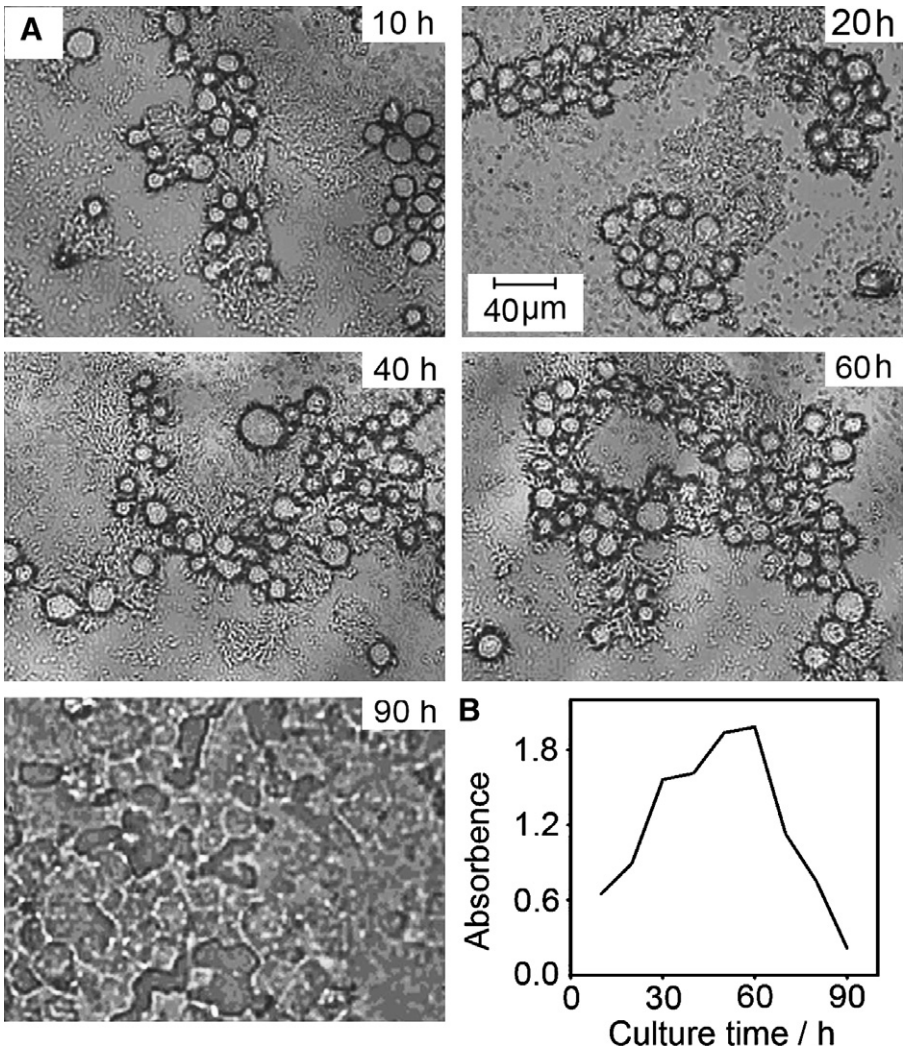

Fig. 4. Photos of K562 cells proliferated on the PANI film at different culture time (A) and MTT assay of K562 cells proliferated on the PANI film (B).

Fig. 4B. The K562 cells proliferated rapidly on the PANI film up to 30 h in culture medium, then the absorbance increased relatively slowly. The absorbance of the collected K562 cells samples showed the maximum value at an incubation time of 60 h, indicating the number of active cells on the PANI film reached the maximum value. After the incubation time longer than 60 h, the absorbance decreased sharply, suggesting the apoptosis of adhered K562 cells, which could be observed on the microscopic image obtained at the incubation time of 90 h.

3.4. Impedance detection of K562 cells

The cell-based sensor was based on the measurement of electron transfer resistance (R_{et}) with $[Fe(CN)_6]^{3-/4-}$ as a redox probe. The probe showed a large resistance at a bare SPCE due to the rather rough surface of the unmodified SPCEs (curve a, inset in Fig. 5A). The assembly of a PANI layer on the electrode surface generated an electroactive and positive-charged film that drastically facilitated the interfacial electron transfer of the negative-charged probe, thus leading to an obvious decrease in R_{et} (curve b). Thus, the presence of conductive PANI improved greatly the electric behaviors of the SPCE. The immobilization of 10^5 K562 cells (in 10 μl 10^7 cells ml^{-1} suspension) on the PANI/SPCE film hindered the access of the redox probe to the electrode due to the resistance of the cell membrane [23], causing an increase in R_{et} (curve c). The change in the R_{et} depended on the amount of the immobilized K562 cells.

With an increasing amount of K562 cells immobilized on PANI/SPCE, the semicircle diameter of Nyquist diagram or the R_{et} of the probe at the obtained cells/PANI/SPCE increased. A linear relationship between the obtained R_{et} and logarithmic value of K562 cells concentration was observed over a range of 10^4–10^7 cells ml^{-1}, with a correlation coefficient of 0.9706 (Fig. 5A). The limit of detection calculated from the slope of the linear plot and the ten times value of standard deviation was 8.32×10^3 cells ml^{-1}, which was more sensitive than that of 4.36×10^5–4.36×10^8 cfu ml^{-1} for an impedance immunosensor for *E. coli* O157:H7 [13]. This proposed method was also much more sensitive than that down to 10^6 cfu ml^{-1} obtained at SPR immunosensors for *salmonella* and *listeria* [24]. The relative standard deviations of R_{et} for three replicative detections of four cell concentrations in the linear range with different PANI/SPCEs prepared in batch were between 3.1% and 4.8% with an average value of 3.9%, indicating acceptable reproducibility of this technique. Due to the broad detection range, low detection limit, and simple fabrication process associated with the proposed cell-based biosensor, it would be well suitable to detection of tumor cells.

3.5. EIS monitoring of cell proliferation on PANI/SPCE

Owing to the disposable manner and mass production of SPCE, electrochemically monitoring of cell proliferation could be achieved conveniently by placing a set of cells/PANI/SPCEs in culture medium in batch, and measuring the Nyquist diagrams at different incubation times, respectively. This way could avoid the effect of cytotoxic electrochemical probe on cell proliferation. As shown in Fig. 5B, with the increasing incubation time of the K562 cells/PANI/SPCE in culture medium, the electron transfer resistance of the redox probe at the produced electrode increased (curves a–e), which obviously resulted from the cell proliferation on the electrode, introducing a greater barrier for electrochemical process due to the increasing amount of cells and the filopodia formed among these cells. In comparison with the R_{et} value on cells/PANI/SPCE prepared by drop-coated method, the proliferated cells on the PANI/SPCE showed much higher R_{et} due to the presence of the filopodia.

The inset in Fig. 5B indicated the electron transfer resistance of the probe increased gradually up to the incubation time of 30 h and then tended to a relatively steady value

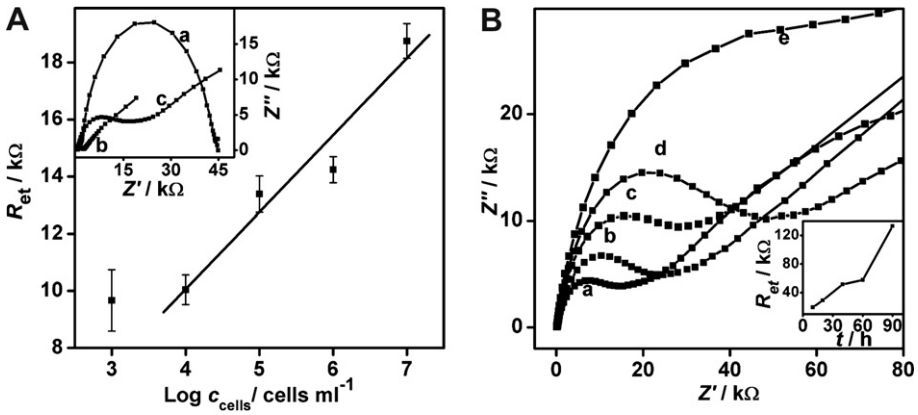

Fig. 5. Linear relationship between electron transfer resistance and logarithm of K562 cells concentration (A), and EIS measurements of K562 cells proliferated PANI/SPCE after incubation for (a) 10, (b) 20, (c) 40, (d) 60 and (e) 90 h (B). Inset in (A) Nyquist diagrams of EIS at (a) bare SPCE, (b) PANI/SPCE and (c) cells/PANI/SPCE and (B) relationship between electron transfer resistance and proliferation time of K562 cells on PANI/SPCE. All data were obtained in 0.01 mol l^{-1} pH 7.4 PBS containing 10 mmol l^{-1} $Fe(CN)_6^{3-/4-}$ and 0.1 mol l^{-1} KCl.

during the incubation time of 30–60 h. After the incubation time of 60 h, the resistance sharply increased due to the apoptosis of cells, which induced the agglomeration of cells on the electrode surface, thus greatly increased the resistance. These changes were consistent with the observation from optical microscopy and MTT assay.

3.6. Stability and reproducibility of the PANI/SPCE

The PANI/SPCE showed very good storage stability with remaining 90% of its initial impedance response after a storage period of two months in a desiccator. The SPCE and PANI/SPCE could be fabricated in batches with acceptable reproducibility of the measured impedance responses. The renewal of the SPCE was unnecessary, thus it was convenient, disposable and low-cost for the electrochemical study of cell proliferation.

4. Conclusions

PANI film is rapidly and conveniently formed by electropolymerization method on SPCE as a support. The designer amine-based polymer is demonstrated to be very effective for cell immobilization and adhesion, which extends the application of SPCE to sensitive cytosensing, electrochemical studying and monitoring of cell proliferation due to the conductivity and biocompatibility of PANI. The results obtained using EIS measurements and MTT assay are in good agreement. The impedance-based sensor can be used for detection of cell concentration with acceptable reproducibility. The advantages of simple and low-cost fabrication, unnecessary renewal, economic and convenient detection of the SPCE can avoid the effect of cytotoxic electrochemical probe on cell proliferation, and are significant to the electrochemical study for cell proliferation. It possesses potential application in miniaturization of the devices in cytology.

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