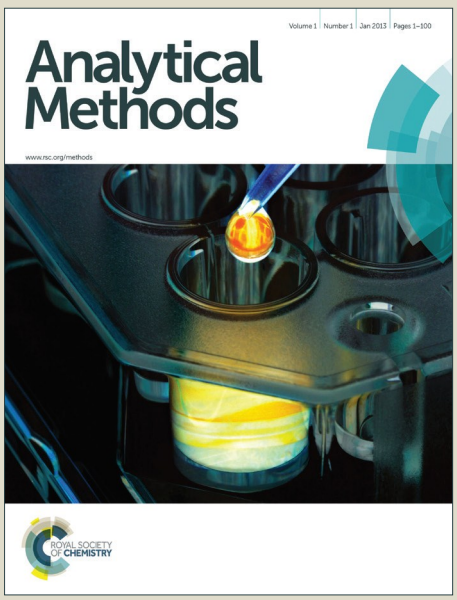
Analytical Methods

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Analytical Methods

PAPER

Voltammetric behavior of HeLa cells on carbon nanotubes/ionic liquid modified electrode for cytotoxicity evaluation of chlorophenols

Received 00th January 2015,
Accepted 00th January 2015

DOI: 10.1039/x0xx00000x

www.rsc.org/

Xiaolin Zhu,[a] Hongwei Qin,[b] Guanlan Wu,[a] Jinlian Li,[c] Xing Yuan,*[a] and Dongmei Wu*[c]

In the present study, the electrochemical behavior of human cervical carcinoma (HeLa) cells on purified-multiwall carbon nanotubes (p-MWCNTs) and ionic liquid (IL) modified glass carbon electrode was studied. Two voltammetric signals of HeLa cells were detected, which were attributed to intracellular xanthine, guanine, hypoxanthine and adenine. The existence of these purines was further verified by high-performance liquid chromatography (HPLC) assay. Based on the variation of the intensity of the two voltammetric signals, the cytotoxicity of 2,4-Dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP) were evaluated, which were also compared with the traditional methyl tetrazolium (MTT) assay. This work is expected to provide a label-free method providing more exact and perfect information to study the purine metabolism, as well as to evaluate the influences of chemicals from different perspectives.

1. Introduction

Chlorophenols (CPs), which are listed as priority pollutants, are widely used in pesticides, germicides, dyestuffs, phenolic resins, etc. They are recalcitrant to biodegradation and pose a serious threat to environment and human.[1-3] Therefore, sensitive and reliable toxicity evaluation of CPs in the environment is greatly important. In recent years, the in vitro toxicity testing technique has been approved as an alternative to whole-animal test.[4-6] Some assays include neutral red assay, methyl tetrazolium (MTT) assay, lactate dehydrogenase leakage assay and deoxyribonucleic acid fragmentation assay have been used in the cytotoxicity evaluation of CPs.[7-9] However, these marking methods have several disadvantages, such as complexity of operation, high cost, and time-consumption. The emerging *in vitro* electrochemical method provides a new sight for scientists owing to its simple instrumentation, rapidness, high sensitivity, and non-toxicity.[10-11] Nevertheless, the mechanism of the electrochemical behavior of cells is poorly studied due to the complexity of intracellular biological characters in the field of cell electrochemistry.

In our previous study, we found that the cell viability was strongly related to intracellular nucleotide metabolism. Thus, the effects of different substances on cell activity could be expressed to the influence on purine bases, which are the intermediate products of nucleotide metabolism. The electrochemical behaviours of human prostate cancer (PC-3) and breast cancer (MCF-7) were studied and the voltammetric signal at approximate + 0.7 V derived from guanine (G) and xanthine (XA) was verified.[12-14] The sensitivities of several drugs were tested using the electrochemical method.[12] Compared with above cells, cervical carcinoma (HeLa) cells are good model organisms.[15-16] Like normal cells, they can produce proteins, express genes, communicate with each other, and are susceptible to infections. They can also proliferate abnormally rapidly, even compared to other cancer cells.[16-19] However, due to the restrictions of current graphene or carbon nanotubes modified electrodes in terms of sensitivity and stability, the electrochemical detection could only be carried out in the potential range from 0.0 to + 0.8 V.[13-15,20] Herein, to the best our knowledge, there is no report on the electrochemical response related to purine metabolism of HeLa cells. If more purine bases can be detected, the toxic effects of different chemicals on nucleotide metabolism will be evaluated more comprehensively by the electrochemical method.

Herein, a more sensitive and stable purified-multiwall carbon nanotubes (p-MWCNTs) and ionic liquid (IL) modified glass carbon electrode (p-MWCNTs/IL/GCE) was prepared. Then the electrochemical behavior of HeLa cells based on p-MWCNTs/IL/GCE was studied. A new signal at + 1.034 V was found for the first time and confirmed from the oxidation of adenine (A) and hypoxanthine (HX). The cytotoxicity of three CPs including 2,4-Dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP) are evaluated based on the two electrochemical signals, and then the results are compared with those

[a] *State Environmental Protection Key Laboratory of Wetland Ecology and Vegetation Restoration, School of Environment, Northeast Normal University, Changchun 130117, P.R. China. E-mail: yuanx@nenu.edu.cn; Fax: +86 431 89165606; Tel.: +86 431 89165600*
[b] *College of Chemistry and Chemical Engineering Bohai University, Jinzhou 121013, P.R. China*
[c] *College of Pharmacy, Jiamusi University, Jiamusi 154007, P.R. China. E-mail: dmwu@jmsu.edu.cn; Fax: +86 454 8618460; Tel.: +86 454 8618461*
† Xiaolin Zhu and Hongwei Qin have equal contribution to this work.

of MTT assay. This work will be beneficial in providing more information of effects on purine metabolism caused by hazardous pollutants.

2. Experimental

2.1 Chemicals and reagents

G, A, XA, HX, trypsinase were purchased from Sigma. Phosphate buffer saline (PBS, pH 7.4, 0.2 M) containing KCl, Na_2HPO_4 and KH_2PO_4 was used. IL ([BMIM]PF_6) were obtained from J&K Scientific Ltd. (China). Three CPs of analytical grade were purchased from Sigma. For the experiments, several serial dilutions of CPs stocks were prepared in the culture medium to concentrations of 199.6, 319.4, 511.0, 817.6, 1308.2 μM for 2,4-DCP, 49.0, 85.7, 150.0, 262.5, 459.4 μM for 2,4,6-TCP and 29.3, 46.9, 75.0, 120.0, 192.0 μM for PCP. All other chemicals were of analytical grade and used without further purification.

2.2 Cell culture and treatment

HeLa cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum, 100 μg/mL penicillin and streptomycin in an incubator (37 °C, 5% CO_2). The DRM-700 counting plate (China) was used for the determination of cell concentrations. Prior to the CPs exposure, the medium was replaced with the mediums with various concentrations of CPs. After a certain incubation time, the culture medium was removed, and the adherent cells were washed with PBS for three times. Next a certain amount of pH 7.4 PBS was added, and finally they were heated in 50 °C water bath for 0.5 h to obtain the HeLa cell suspension.

2.3 Preparation of the p-MWCNTs/IL/GCE

Purification procedures were applied to MWCNTs (diameter: 10-20 nm, Shenzhen Nanotech Port Co. Ltd, China) by refluxing in 30 % HNO_3 at 100 °C for 1 d. They were then separated, washed with double-distilled water, filtered through a 0.45 μm filter membrane and dried at 120 °C to obtain the p-MWCNTs. The GCE was polished with 1.0, 0.3 and 0.05 μm alumina slurry, followed by rinsing thoroughly with double-distilled water and ethanol in an ultrasonic bath. Then the GCE was allowed to dry at room temperature. 1 mg p-MWCNTs and 20 μL IL were ground together. A portion of p-MWCNTs/IL was coated on the GCE to fabricate p-MWNTs/IL/GCE. For comparison, a MWCNTs/GCE was prepared according to our group's previous work.[12] Prior to use, all the electrodes were scanned in pH 7.4 PBS for twenty cycles to obtain stable background.

2.4 Electrochemical experiments

In this work, the p-MWCNTs/IL/GCE (working electrode), the Pt wire (counter electrode), and the Ag/AgCl (reference electrode) were conducted on a LK2005 electrochemical workstation (Lanlike Instruments, China) for electrochemical measurements. After each measurement, the p-MWCNTs/IL/GCE was renewed by scanning for five times in pH 7.4 PBS from 0.0 to + 1.1 V. 480 s were selected as the best accumulation time for the electrochemical experiments. The cytotoxicity of CPs was calculated from the formula:

$$\text{Cytotoxicity} = [(i_{blank} - i_{CP})] / i_{blank}] \ 100\ \% \qquad (1)$$

where i_{blank} and i_{CP} are the peak current of the HeLa cell suspension in the absence and presence of CP. The IC_{50} values were obtained with the Probit analysis.[21]

2.5 MTT assay

1.2×10^4 cells/mL HeLa cells in 200 μL of either the medium alone or the medium containing different CPs were added to the 96 well microtitre plates. Plates were incubated at 37 °C for 30 h, then 20 μL of 5 mg/mL MTT (Sigma) was added to the wells. Four hours later, the medium containing MTT was removed and 150 μL sodium dodecyl sulfate was added. The absorbance was recorded at 490 nm using the microplate spectrophotometer system. The cytotoxicity of CPs was calculated from the formula:

$$\text{Cytotoxicity}=[(A_{blank} - A_{CP}) / A_{blank}] \ 100\ \% \qquad (2)$$

where A_{CP} is absorbance of CP-treated well and A_{blank} is absorbance of control well.

2.6 High-performance liquid chromatography (HPLC) measurements

Prior to HPLC, the proteins in the HeLa cell suspension were removed by subsiding the denatured protein at 50 °C for 0.5 h. The cell suspension was filtrated through a 0.22 μM filter after a centrifugation at 14000 × g for about 50 min. An HPLC system (Agilent 1100 series) equipped with an Ascenis RP-Amide column (4.0 × 250 mm), a quaternary pump, an inline degasser and a diode array detector (DAD) was used for the analysis of samples. The mobile phase with 0.02 M KH_2PO_4 was run at 1.0 mL/min. The injection volume was 20 μL for all samples.

2.7 Statistical analysis

Each experiment was carried out at least three times. Results were expressed as mean ± standard deviation (SD). Data from all experiments were analyzed with Statistical Product and Service Solutions (SPSS) 19.0 and Origin 8.0. Statistical differences were evaluated by using student's *t*-test. *p*-value of less than 0.05 was considered statistically significant.

3. Results and discussion

3.1 Voltammetric behavior of HeLa cell suspension

Fig. 1 shows the cyclic voltammograms (CVs) of HeLa cell suspension. No peak is detected with the GCE in HeLa cell suspension (Fig. 1a) and with the p-MWCNTs/IL/GCE in PBS (Fig. 1b). However, the background current of the p-MWCNTs/IL/GCE is higher than that of the GCE, which reflects its more effective surface area. With the p-MWCNTs/IL/GCE in HeLa cell suspension, two anodic peaks at + 0.720 V and + 1.034 V were observed obviously (Fig. 1d). Additionally, the oxidation potentials shift to less positive ones and the currents are greater than those of the MWCNTs/GCE (Fig. 1c) used commonly in our previous study.[12] Moreover, compared with unpurified-MWCNTs/IL/GCE (Fig. 1e), the p-MWCNTs/IL/GCE exhibits higher electrocatalytic activities owing to hydrophilic functional groups such as COOH and OH obtained from the purification. These results imply that the p-MWCNTs/IL film possess excellent electrocatalytic activities towards electroactive species in the HeLa cell suspension, and promote electron-transfer rate on the surface of the electrode. No reduction

peak is found in the reverse scan, which indicates that electrooxidation reaction is an irreversible process. Compared with the first scan (curve a in the inset of Fig. 1), the peak current reduces greatly in the second scan (curve b in the inset of Fig. 1) possibly due to the products of oxidation reaction that has blocked the p-MWCNTs/IL/GCE surface.[15] Hence the first scan was chosen for the voltammetric analysis.

The possible biochemistry mechanism of the two voltammetric signals is studied. As shown in Fig. 2A and Fig. 2B, the peak potentials of XA (+ 0.718 V) and G (+ 0.722 V) are very close, as well as those of A (+ 1.029 V) and HX (+ 1.035 V). The irreversible anodic peaks of the mixture of XA, G, A and HX are at + 0.719 V and + 1.031 V, which are almost the same as those of the HeLa cell suspension (+ 0.720 V and + 1.034 V) (Fig. 2C). The negligible discrepancies in peak potentials between the cell suspension and the mixture of G/XA and A/HX are probably ascribed to the weak pH increase caused by the bases secreted into the cell suspension.[22] The above results show that the peaks could be caused by the oxidation of G/XA and A/HX in HeLa cell suspension.

3.2 HPLC confirmation of purine bases in HeLa cell suspension

The HPLC method was employed to further verify the existence of the four purine bases in HeLa cell suspension. As shown in Fig. 3, the chromatogram indicates that HX, G, XA and A have the retention time of 9.823, 10.80, 11.77 and 14.90 min, respectively (Fig. 3A). They are in good agreement with the four chromatographic peaks of the HeLa cell suspension at 9.864, 10.75 11.79 and 14.89 min respectively (Fig. 3B), which illustrates the existence of the above four purine bases secreted into the HeLa cell suspension.

3.3 Description of HeLa cell growth

G, XA, A and HX are metabolic intermediates of intracellular guanine nucleotide (GMP), xanthine nucleotide (XMP), adenine nucleotide (AMP) and inosinic acid (IMP) as interpreted in Fig. 4. The high cell viability could accelerate the intracellular metabolic process and then more G, XA, A and HX were metabolized. Thence, the electrochemical measurement of purines which act as intracellular signaling molecules, could be selected to reflect the cell viability.

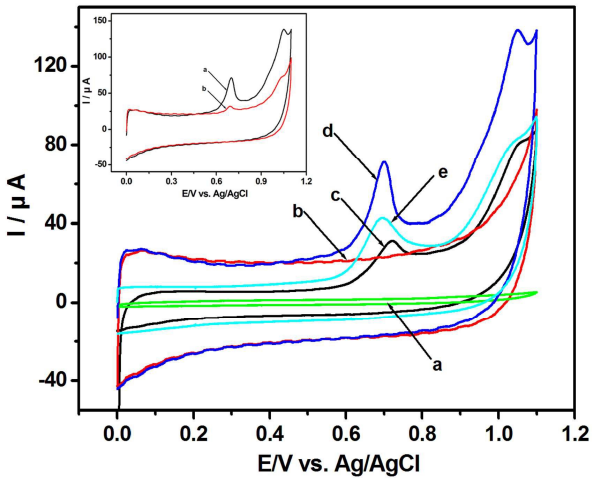


Fig. 1 CVs of (a) the GCE in HeLa cell suspension, (b) the p-MWCNTs/IL/GCE in pH 7.4 PBS, (c) the MWCNTs/GCE in HeLa cell suspension, (d) the p-MWCNTs/IL/GCE, and (e) the unpurified-MWCNTs/IL/GCE in HeLa cell suspension. Inset: CVs of HeLa cell suspension with the p-MWCNTs/IL/GCE at (a) the first scan, (b) the second scan. Cell concentration: 4.0×10^6 cells/mL; scan rate: 50 mV/s.

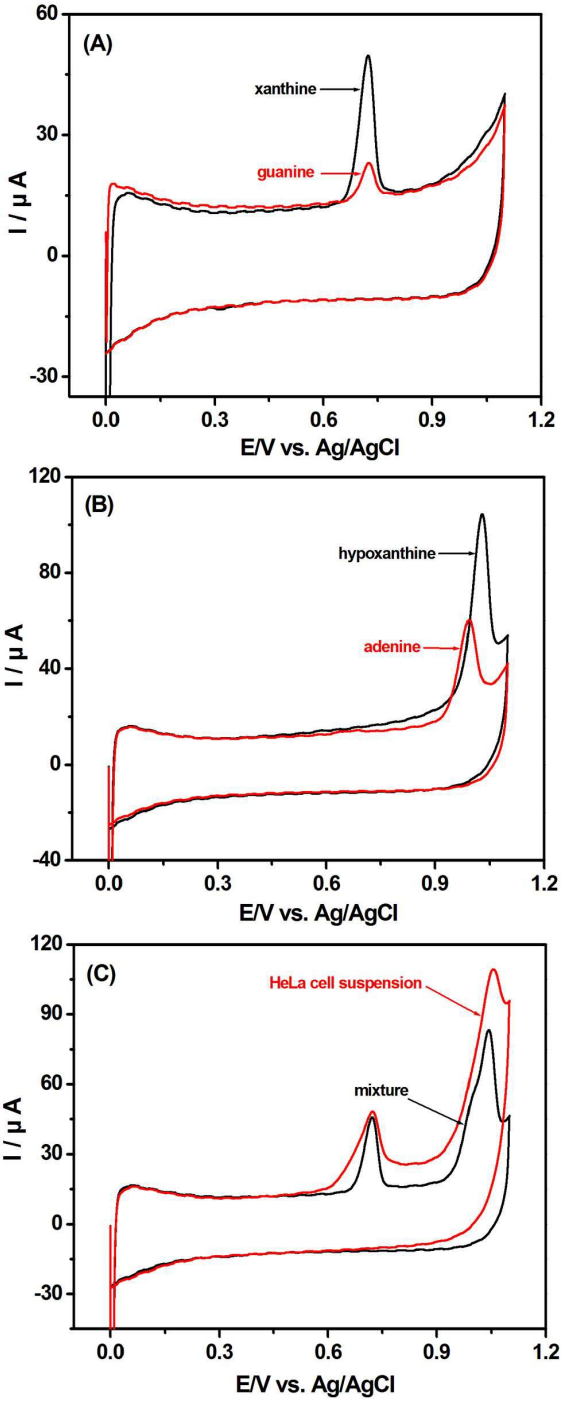


Fig. 2 CVs of p-MWNTs/IL/GCE in (A) 6.0 μg/mL G and 3.0 μg/mL XA, (B) 3.0 μg/mL HX and 3.0 μg/mL A, (C) mixture of 3.0 μg/mL

G, 1.5 μg/mL XA, 1.5 μg/mL HX and 1.5 μg/mL A, and the heat-treated HeLa cell suspension. Cell concentration: 3.2×10^6 cells/mL; scan rate: 50 mV/s.

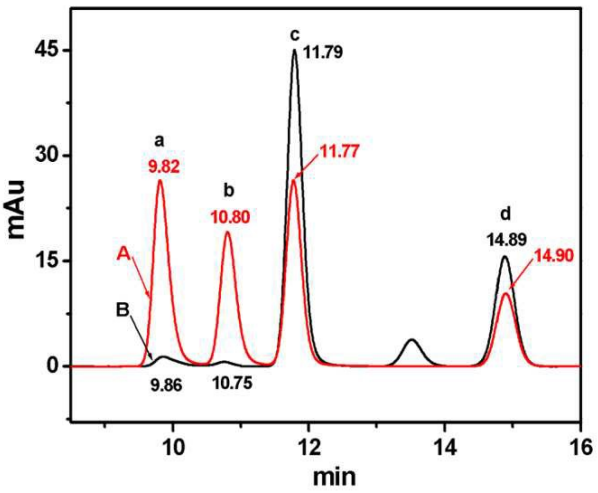


Fig. 3 Chromatograms of (A) the mixture of 50.0 μg/mL HX (a), G (b), XA (c) and A (d), and (B) the HeLa cell cytoplasm eluent. Cell concentration: 7.2×10^6 cells/mL.

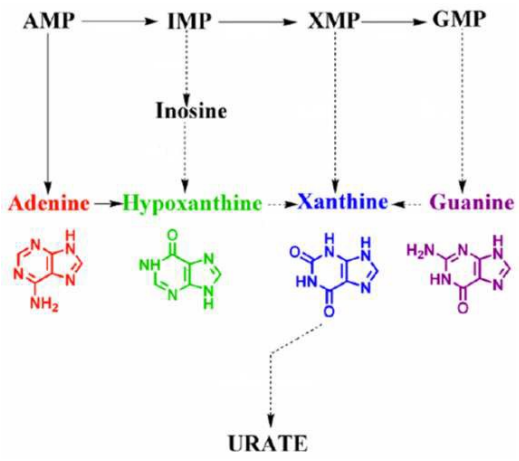


Fig. 4 Catabolism of G, XA, HX and A nucleotides intracellular.

HeLa cells with the same concentration were cultured under same conditions. They were harvested and heated for detection every 6 h. Fig. 5A show the cell growth curves depicted by the two electrochemical signals of HeLa cell suspension. The peak currents increase gradually in 30 h because of the proliferation of HeLa cells, and then decrease sharply, owing to the apoptosis of cells attributed to the nutrient deficiency. The results are similar to the findings of cell counting method (Fig. 5B). Therefore, both signal I and II could be used to describe the growth of HeLa cells.

3.4 Cytotoxicity evaluation of 2,4-DCP, 2,4,6-TCP and PCP

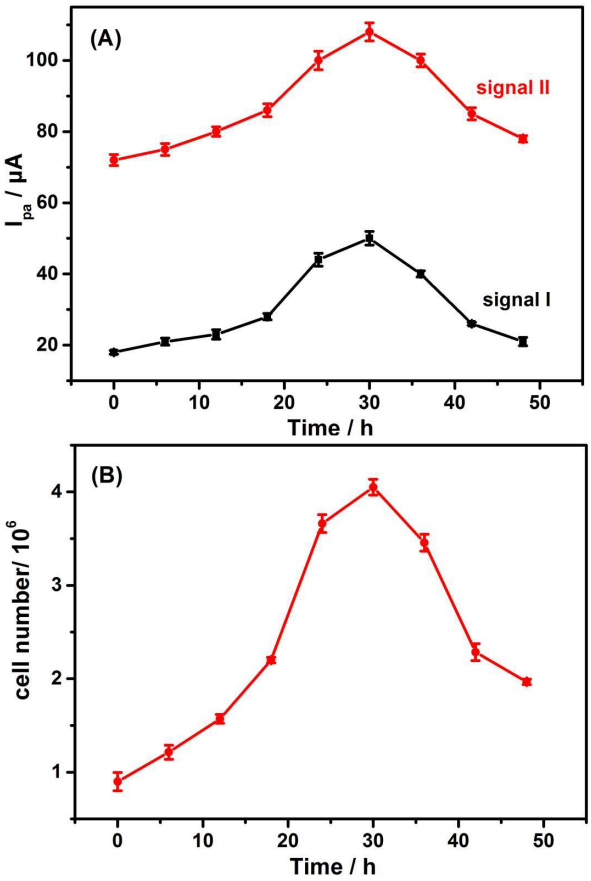


Fig. 5 Growth curves of the HeLa cells described by (A) the electrochemical method-signal I (a) and signal II (b), and (B) the cell counting method.

The voltammetric behavior of HeLa cell suspension after the cells were treated with different CPs was studied. All the peak currents of treated CPs are less than those of the control, which indicates that the three CPs have inhibitory effects on HeLa cells. The cytotoxicity reached to the maximum after CPs were added to the cell culture medium for 30 h. Therefore, 30 h was chosen as the optimal CPs-treated time in the experiment.

Fig. 6 shows the cyclic voltammograms of HeLa cells treated by CPs with various concentrations for 30 h. The peak currents of CP-treated groups are less than those of the control groups. All the treatment groups had significant differences compared with the control group. The oxidation peak currents depend on the concentration of CPs. As the concentration of 2,4-DCP, 2,4,6-TCP and PCP increases, the peak currents reduce. The cytotoxicity of CPs can act in several ways including inducing chromosome breakage and aneuploidy, damaging the cell membrane and cytoplasm and inhibiting ATP synthesis.[23-26] The above results could induce the reduction of cell viability, and reduce the peak currents attributed to the four purine bases, which indicates the change of the physiological characteristics of HeLa cells.

Analytical Methods

PAPER

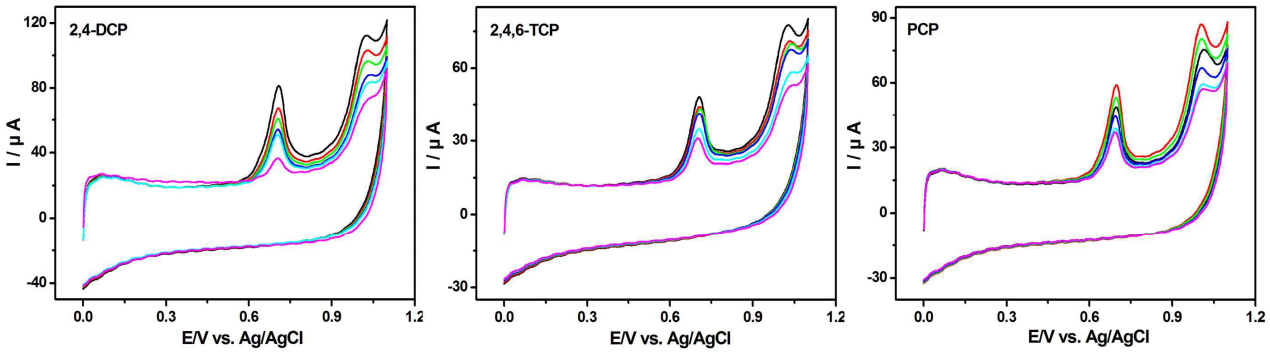


Fig. 6 CVs of HeLa cell suspension treated by three CPs with various concentration. Cell inoculation concentration: 6.0×10^5 cells/mL; treated time: 30 h; scan rate: 50 mV/s.

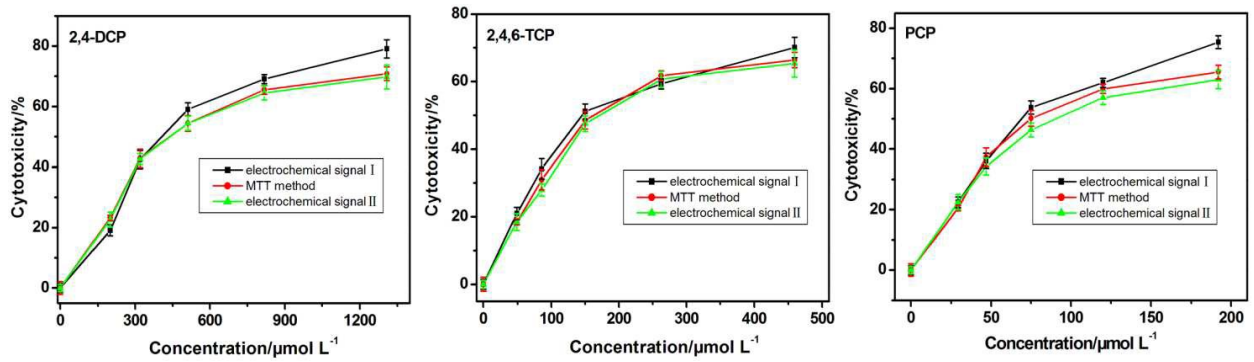


Fig. 7 Cytotoxicity curves of 30 h exposure of the HeLa cells to three CPs obtained with the electrochemical method and the MTT assay. Cell inoculation concentration for the electrochemical method and MTT assay: 6.0×10^5 cells/mL and 1.2×10^4 cells/mL.

Fig. 7 displays the cytotoxicity curves of HeLa cells exposed to 2,4-DCP, 2,4,6-TCP and PCP for 30 h. The cytotoxicity is linear with the negative logarithm concentration of CP. The IC_{50} values obtained by the electrochemical signal I and signal II are 451.8 and 495.8 μM for 2,4-DCP, 172.5 and 198.5 μM for 2,4,6-TCP, and 75.91 and 96.40 μM for PCP, respectively. The obtained cytotoxic tendency is PCP > 2,4,6-TCP > 2,4-DCP, which is consistent withthe conclusions in other literatures and also confirms a correlation between the cytotoxicity of CPs and the number of substituted chlorine atoms.[22,27] However, the IC_{50} obtained by the signal I is lower than that of signal II, which may reflect that 2,4-DCP, 2,4,6-TCP and PCP have greater impacts on xanthine/guanine than that on hypoxanthine/adenine. The results indicate the different effects of CPs on diverse levels of intracellular purine metabolism. Compared with the previous one-signal electrochemical method, the two electrochemical signals can provide more information about effects of CPs with different parameters, which also has significance for research of physiological process about purine metabolism. Moreover, the MTT assay was compared with the results obtained by the electrochemical method based on HeLa cells. The IC_{50} values obtained by MTT assay are 484.2, 187.9 and 86.81 μM for 2,4-DCP, 2,4,6-TCP and PCP, respectively. The toxicity orders are consistent with those by the electrochemical test. The results suggest that the electrochemical method based on intracellular purine metabolism of HeLa cells was reliable to assess the cytotoxicity of CPs. Moreover, compared with the traditional MTT assay, the label-free electrochemical method is much easier and rapider. The electrochemical detection process only needs 0.5 h, while the MTT assay takes about 5 h.

Finally, the repeatability and reproducibility of p-MWCNTs/IL /GCE were investigated by twenty successive voltammetric measurements. The relative standard deviation (RSD) of 1.0×10^6 cells/mL HeLa cell suspension is 4.31%. Besides, six p-MWCNTs

/IL/GCEs were independently fabricated to be applied to 1.0×10^6 cells/mL HeLa cell suspension. Statistical differences among the results were tested by student's *t*-test. The p-value is 0.155, which indicates there is no significant change among the six p-MWCNTs /IL/GCEs ($p > 0.05$). The results demonstrate that the p-MWCNTs /IL/GCE possesses good repeatability and reproducibility. Additionally, the linear detection ranges of p-MWCNTs/IL/GCE are 0.5 to 220.0 μM for G, 0.25 to 200.0 μM for XA, 0.5 to 250.0 μM for A, 0.5 to 250.0 μM for HX, and 5.0×10^3 to 4.5×10^6 cells/mL for HeLa cells, respectively. The lower detection limits are 0.1 μM, 0.03 μM, 0.04 μM, 0.04 μM and 1.0×10^3 cells/mL.

4. Conclusion

In summary, the biochemical mechanism of the voltammetric response for HeLa cell suspension on p-MWCNTs/IL/GCE was explained. The cytotoxicity of 2,4-DCP, 2,4,6-TCP and PCP were evaluated by the two voltammetric signals. Further, the results were compared with those of the conventional MTT assay. The electrochemical assay developed here is rapider, simpler and can be usefully employed to assess more thoroughly the cytotoxicity of CPs. This study provides a basis for future research aiming to evaluate the effects of different substances based on purine metabolism.

Acknowledgments

We gratefully acknowledge the National Natural Science Foundation (NNSF) of China (51178092 and 81273132), the Fundamental Research Funds for the Central Universities (14ZZ1517), Nature Science Foundation of Heilongjiang Province (D201005 and D201016 a), Scientific Research Fund of Heilongjiang Provincial Education Department (12511542) and the PhD Start-up Fund of Bohai University (bsqd201429).

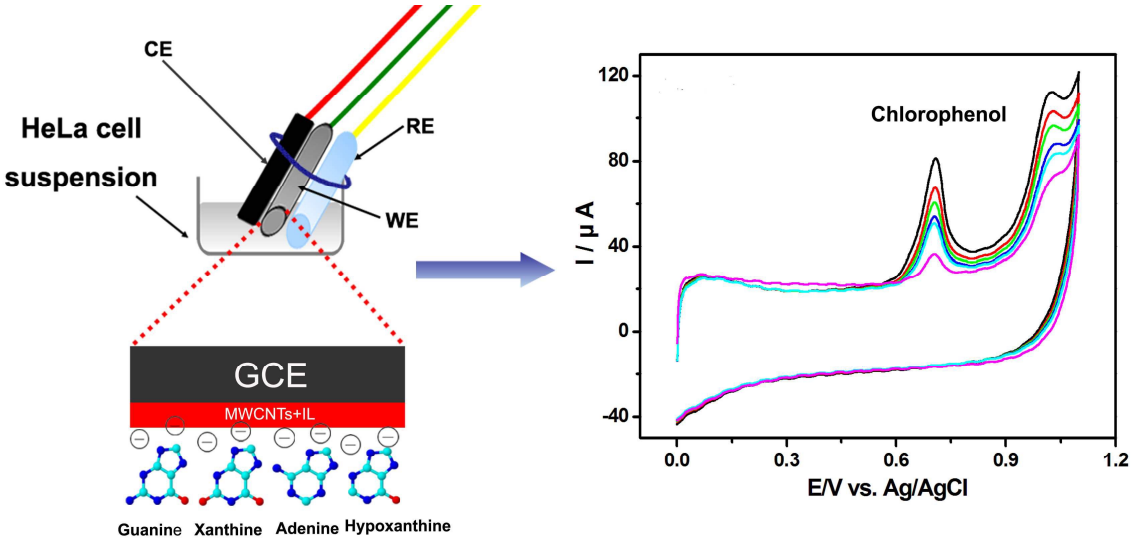
References

1 S.S. Gupta, M.Stadler, C.A. Noser, A. Ghosh, B. Steinhoff, D. Lenoir, C. P. Horwitz, K.W. Schramm and T.J. Collins, *Science*, 2002, **296**, 326-328.
2 C.S.Evans and B. Dellinger, *Environ. Sci. Technol.*, 2005, **39**, 122-127.
3 A. Karci, *Chemosphere*, 2014, **99**, 1-18.
4 M. Davoren and A.M. Fogarty, *Toxicol. in Vitro*, 2006, **20**, 1190-1201.
5 X. Liu, J. Chen, H. Yu, J. Zhao, J.P. Giesy and X. Wang, *Chemosphere*, 2006, **64**, 1619-1626.
6 G.O. Rankin, A.Sweeney, C. Racine, T. Ferguson, D. Preston and D.K. Anestis, *Chem. Biol. Interact.*, 2014, **222**, 126-132.
7 A. Castaño and M.J. Gómez-Lechó, *Toxicol. in Vitro*, 2005, **19**, 695-705.
8 J. Chen, J. Jiang, F. Zhang, H. Yu and J. Zhang, *Cell Boil. Toxicol.*, 2004, **20**, 183-196.
9 H. Saito, J. Koyasu, T. Shigeoka and I. Tomita, *Toxicol. in Vitro*, 1994, **8**, 1107-1112.
10 L. Ding, D. Du, J. Wu and H. Ju, *Electrochem. Commun.*, 2007, **9**, 953-958.
11 D.L. Robinson, T.J. Volz, J.O. Schenk and R. M. Wightman, *Alcohol. Clin. Exp. Res.*, 2005, **29**, 746-755.
12 D.M. Wu, G.L. Fu, J.T. Wang, L.Y. Ge, J.H. Zhu, X. Yuan and J.G. Liu, *Electrochem. Commun.*, 2011, **13**, 623-626.
13 H. Qin, Q. Gao, H. Niu, Z. Wang, X. Zhu, J. Li, X. Yuan and D. Wu, *Analyst*, 2013, **138**, 3372-3375.
14 X. Guo, Q. Wang, J. Li, J. Cui, S. Zhou, S. Hao and D. Wu, *Biosens. Bioelectron.*, 2015, **64**, 594-596.
15 X. Zhu, H. Qin, J. Liu, Z. Zhang, Y. Lu, X. Yuan and D. Wu, *J. Hazard. Mater.*, 2014, **271**, 210-219.
16 M. Ivanković, A. Ćukušić, I. Gotić, N. Škrobot, M. Matijašić, D. Polančec and I. Rubelj, *Biogerontology*, 2007, **8**, 163-172.
17 B. Ekwall, "Toxicity to HeLa cells of 205 drugs as determined by the metabolic inhibition test supplemented by microscopy," *Toxicology*, 1980, **173**, 273-295.
18 R.A. Garduño, F.D. Quinn and P.S. Hoffman, *Can. J. Microbiol.*, 1998, **44**, 430-440.
19 S. Patel, N. Gheewala, A. Suthar and A. Shah, *Int. J. Pharm. Pharm. Sci.*, 2009, **1**, 38-46.
20 K. Chen, J. Chen, M. Guo, Z. Li and S. Yao, *Electroanal.*, 2006, **18**, 1179-1185.
21 K. Thomulka, C. Abbas, D. Young and J. Lange. *B. Environ. Contam. Tox.*, 1996, **56**, 446-452.
22 C.Y. Chen and J.H. Lin, *Chemosphere*, 2006, **62**, 503-509.
23 M. J. Armstrong, S. M. Galloway and J. Ashby, *Mutat. Res. Lett.*, 1993, **303**, 101-108.
24 P. Fernández Freire, V. Labrador, J.M. Pérez Martín and M.J. Hazen, *Toxicology*, 2005, **210**, 37-44.
25 T.T. Liao, Y.L. Shi, J.W. Jia, R.W. Jia and L. Wang, *J. Hazard. Mater.*, 2010, **179**, 1055-1064.
26 Y.J. Wang, Y.S. Ho, J.H. Jeng, H.J. Su and C.C. Lee, *Chem. Biol. Interact.*, 2000, **128**, 173-188.
27 T. Tiensing, N. Strachan and G.I. Paton, *J. Environ. Monit.*, 2002, **4**, 482-489.

Graphical Abstract

Voltammetric behavior of HeLa cells on carbon nanotubes/ionic liquid modified electrode for cytotoxicity evaluation of chlorophenols

Xiaolin Zhu[a], Hongwei Qin[b], Guanlan Wu[a], Jinlian Li[c], Xing Yuan[a,*] and Dongmei Wu[c,*]



Two voltammetric signals based on the purine metabolism of human cervical carcinoma (HeLa) cells on a sensitive p-MWCNTs/IL/GCE was discovered. The cytotoxicity of three chlorophenols was tested with this double-signal electrochemical method.