J Appl Electrochem (2008) 38:1735–1741
DOI 10.1007/s10800-008-9623-2

ORIGINAL PAPER

# Adenosine 5′-triphosphate incorporated poly(3,4-ethylenedioxythiophene) modified electrode: a bioactive platform with electroactivity, stability and biocompatibility

**Y. H. Xiao · C. M. Li · M.-L. Toh · R. Xue**

Received: 31 March 2008 / Accepted: 16 June 2008 / Published online: 3 July 2008


**Abstract** Poly(3,4-ethylenedioxythiophene) (PEDOT) was potentiostatically polymerized onto gold electrode with adenosine 5′-triphosphate (ATP), an important biomolecule, as the counterion. Essential parameters to affect the impedance of the resultant polymer were studied in detail. Results show that 1.0 V, 0.1 M and 2.2 mC is the optimal deposition potential, dopant concentration and quantity of the passing charges, respectively. Surface topography was studied by AFM and the relationship between impedance and topography was discussed. Spontaneous release of ATP from PEDOT matrix was examined using UV-visible spectroscopy and impedance variation of the polymer modified electrode was monitored with electrochemical impedance spectroscopy. The results indicate that ATP could be strongly bound with the polymer in the incubation medium and thus could realize its role as a cell binder in its implantable application. In vitro test further demonstrates that PC12 cell could adhere to and grow on the PEDOT/ATP modified electrode and cell attachment percentage was much higher than that of non-biomolecule doped PEDOT modified electrode. Moreover, stability of PEDOT/ATP in the biological reducing agent of glutathione (GSH) was much better than that of polypyrrole/ATP. Our work demonstrates that PEDOT/ATP modified electrode can be of a promising bioactive platform to be used for implantable neural recording devices due to its great stability and biocompatibility.

**Keywords** PEDOT · ATP · Modified electrode · Stability · Biocompatibility

Y. H. Xiao · C. M. Li (✉) · M.-L.Toh · R. Xue
School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 637457, Singapore
e-mail: ecmli@ntu.edu.sg

Y. H. Xiao · C. M. Li
Center for Advanced Bionanosystems, Nanyang Technological University, Singapore 637457, Singapore

Y. H. Xiao
Jiangsu Key Laboratory of Bio-Functional Materials, Nanjing Normal University, 210097 Nanjing, China

## 1 Introduction

Over the past decades, modification of neural electrodes including microwires, microshafts, microelectrode arrays and micromachined planar electrodes have been extensively investigated to achieve better performance in their applications [1–5]. These electrodes are particularly used in the stimulation and recording of signals from neural cells. Hence, the stability in biological environment and the affinity to neurons of the surface materials become the critical factors. Conducting polymers, which have inherent ion/electron transfer property could establish an intermediate layer (bridge) between the brain tissue and metal electrode substrate, in both mechanical and electrochemical respects. Of these polymers, polypyrrole (PPy) has been selected to be the modifiers of electrodes due to its aqueous-compatibility, ease of preparation, beneficial chemical properties, and tunable surface morphology [6–9]. In recent years, another type of conducting polymer, poly(3,4-ethylenedioxythiophene) (PEDOT), has been introduced into this field. Studies on its electrochemical synthesis, electrochemical properties, morphology control and applications have grown enormously [10–13]. In comparison with PPy, PEDOT was

found to be much more stable under constant polarization conditions and able to retain its conductivity even after storage at high temperature [14, 15]. The better performance is derived from its high regioregularity, which gives a product that lacks the presence of undesired $\alpha,\beta$- and $\beta,\beta$-coupling within the polymer backbone [16]. Thus, it has become a promising candidate to modify metal electrodes for the applications of implantable devices, especially for long term use due to its stability [17].

It has been reported that by electrochemical polymerization, dopants (counterions) could catalyze the synthesis and provide a means for changing the physical and biological properties of the conducting polymer [18]. Large or strongly charged dopants have an inclination to be strongly bound and do not diffuse away after long periods of incubation in media [19]. Strong binding of a biomolecule dopant in a polymer is essential for a polymer-modified sensor electrode, in which the dopant gives the electrode affinity to a cell to be monitored. Good stability and immobilization of the dopant in the polymer matrix render the modified electrode an excellent substrate for cell attachment for long time use. In this paper we chose PEDOT as the conducting polymer and adenosine 5′-triphosphate (ATP) as the counterion (dopant). ATP is an important trophic factor, which is co-stored and co-released at central and peripheral cholinergic synapses and has long been recognized to be an activator of sensory nerves [20, 21]. Additionally, it is thought to play some roles as a neurotransmitter in regulating excitability of neurons [22]. Hence it is of great importance to incorporate ATP into PEDOT for expecting excellent performance as a neural implantable electrode material.

Biocompatibility with cells as well as incorporation of bioactive molecules into a matrix are especially attractive for devices used in contact with biological tissue. Our work here focused on PEDOT electrosynthesis with ATP incorporation. Different deposition parameters were employed to optimize the electrochemical properties of the resultant polymer. UV-visible absorbance studies were carried out to investigate the stability of ATP in the PEDOT/ATP matrix. Cell culture was performed with neuron-like rat pheochromocytoma cells (PC12) on the PEDOT/ATP modified electrode and the cell adhesion was tested and analyzed. It is also important that polymer and dopant modified devices be stable in the presence of biologically relevant reducing agents such as glutathione (GSH). Good stability of the material in the reducing agent environment would benefit its long-term application as a modifier of the implanted devices. Impedance variation of the PEDOT/ATP modified electrode with immersion time in GSH was studied. In order to illustrate the advantage of PEDOT, PPy was employed for comparison.

## 2 Experimental

EDOT monomer with a molecular weight of 142.15 was obtained from Aldrich and purified via vacuum distillation and kept refrigerated at 4°C under nitrogen before use. ATP disodium salt (>99%) was purchased from Sigma-Aldrich and used without further purification. All other chemical reagents were received from Aldrich and used as received. PC12 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and were cultured in a Dulbecco's modified Eagle medium (DMEM, Gibco, Carlsbad, CA, USA). All solutions were prepared with double-distilled deionized water (Milli-Q, Millipore Inc.).

A CHI 760B Electrochemical Station (Texas, USA) was employed for the electrosynthesis of PEDOT and electrochemical measurements as well. Gold disk electrodes with diameter of 2 mm were polished with 0.3 and 0.05 μm alumina powder, rinsed with ethanol and water and cleaned in an ultrasonic bath for 20 min before use. Surface topography of the PEDOT/ATP film was investigated with AFM (Dimension 3100 SPM, Veeco, USA) and high resolution surface images were produced. In AFM characterization, the tapping mode with a silicon probe (RTESP, Veeco, USA) over scan sizes of 5 μm and the scan rate of 0.20 Hz was used. Characteristic section analyses were obtained by AFM software. UV-visible spectrophotometer (Shimadzu, UV-2450, Japan) was employed to study the release of ATP from PEDOT. Optical images were obtained using a Leica DFC300 FX digital camera and collected as JPG images.

PEDOT/ATP was electrosynthesized potentiostatically from monomer solution containing 0.01 M EDOT and 0.1 M ATP at 1.0 V for varying deposition time. The monomer solution was purged with nitrogen stream for 20 min prior to electrodeposition. The gold disk electrode and gold coated silicon wafer were used for electrochemical studies and cell culture, respectively. Linear Sweeping Voltammetry (LSV) was conducted in 0.01 M EDOT and 0.1 M ATP aqueous solution from 0 to 1.8 V, with a scan rate of 50 mV $s^{-1}$. Electrochemical impedance spectroscopy (EIS) was conducted in 0.01 M phosphate buffer saline (PBS, pH 7.4). 5.0 mV ac sinusoid signal was applied as the input perturbation and dc bias potential was set at 0.0 V. The impedance measurements were carried out in a frequency range of 10–$10^5$ Hz. A cleaned gold electrode/polymer coated electrode, a platinum wire, and Ag/AgCl (saturated KCl) were used as the working, auxiliary, and reference electrode, respectively for electrosynthesis and impedance measurements. After polymerization under the conditions described above, the PEDOT/ATP coated electrode was submerged in 0.1 M NaCl bath solution. The coated electrode was removed from the bath solution at time intervals

and impedance spectroscopy was examined. In addition, an aliquot (1 μL) was taken from the bath solution and tested in a range of 200–300 nm by using UV spectrometer. For stability investigation, the PEDOT/ATP coated electrode was immersed in 10 mM GSH solution for a certain time. The electrode was removed from GSH at intervals for impedance measurements. In order to demonstrate the better stability of PEDOT over PPy, PPy/ATP was electropolymerized and the coated electrode was subjected to the same experiments.

Before electrosynthesis, the gold plated silicon wafers were subjected to a base cleaning procedure for removing organic substance adsorbed on the surface that could be harmful to cells. After electrochemical deposition of PEDOT/ATP, the wafers were thoroughly rinsed with water and then soaked in copious water for 8 h. Finally, the PEDOT/ATP coated wafer and the PEDOT/$LiClO_4$ coated wafer (for comparison purpose) were placed in a culture dish and sterilized under ultraviolet light overnight in a laminar flow hood. PC12 cells were grown in DMEM supplemented with 10% horse serum and 5% fetal bovine serum in a humidified incubator with 5% $CO_2$ at 37°C. The cell suspension was transferred onto the coated electrode surface for cell attachment and cell growth monitoring.

## 3 Results and discussion

PEDOT/ATP was electrodeposited onto the gold electrode surface under potentiostatic mode. Figure 1a shows that no electrochemical reaction occurred in EDOT/ATP aqueous solution in a deposition potential range lower than 0.8 V. The current initially increased when the potential was higher than 0.9 V and a poorly defined peak appeared around 1.0 V, indicating an electrochemical reaction happened. With further increase of the potential, a well defined peak emerged at 1.25 V. From previous study [23], we know that the first poorly defined peak corresponds to the polymerization of the monomer and the second prominent peak is assigned to the over-oxidation of the polymer. LSV in pure EDOT solution (Fig. 1b) as well as that in pure ATP solution (Fig. 1c) are shown for comparison to further demonstrate the conclusion. Thus the suitable potential for PEDOT/ATP deposition should be between the two peak potentials. In our experiments, 1.0 V was chosen as the deposition potential.

The effect of polymerization potential on the deposition of PEDOT/ATP was further investigated in a potential ranged from 0.8 to 1.2 V. When the potential was 0.8 V, the current flowing in the solution was in the order of $10^{-9}$ A, and the current remained unchanged even after 1 h without formation of the polymer film on the electrode surface, which is similar to that in a buffer solution. A similar phenomenon was observed for the polymerization at 0.9 V, except that the current flowing was in the order of $10^{-8}$ A. On the contrary, black films could be easily obtained when the potential was higher than 1.0 V.

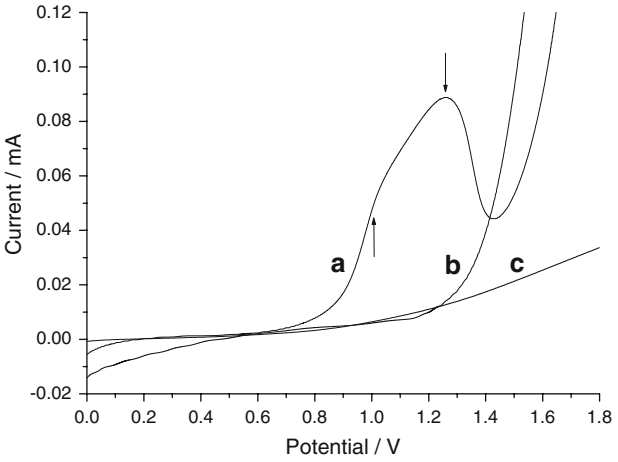


**Fig. 1** LSV obtained from different aqueous solution. The scan rate was 50 mV $s^{-1}$ (**a**) 0.01 M EDOT and 0.1 M ATP solution; (**b**) 0.01 M EDOT solution; (**c**) 0.1 M ATP solution

This is in agreement with our observation from LSV. In this work, we measured the impedance at 1 kHz to evaluate the performance of the coated electrodes since the frequency is characteristic of neural biological activity.

Figure 2A shows that the deposition potential had a significant effect on the impedance at 1 kHz. The higher the deposition potential, the higher is the impedance magnitude of the synthesized polymer film. From Fig. 1 we know that the lowest potential at which PEDOT/ATP could be obtained is 1.0 V. At higher potentials the polymer film tends to be over-oxidized, resulting in an increased resistance. The results demonstrate that the electronic properties of the PEDOT/ATP are dependant on its polymerization potential.

The concentration of dopant is also an important factor to affect the property of the conducting polymer. PEDOT/ATP was electrosynthesized with different ATP concentrations from 0.05 to 0.2 M. EIS was examined and the relationship between the ATP concentration and impedance magnitude at 1 kHz was plotted as in Fig. 2B.

The curve of resistance versus ATP concentration exhibits an upside down volcanic shape indicating that 0.1 M gives a minimum resistance and is the optimal concentration of ATP used in our study. The phenomenon reveals the reaction mechanism between PEDOT and ATP. As it is well known, conducting polymers exist as polycations in their conductive (oxidized) state. 0.1 M of ATP provides equal negative charges to the polycations of PEDOT. When ATP is not sufficient, not all the polymer converts to its oxidized state. On the other hand, when the ATP concentration is too high, the polycations are

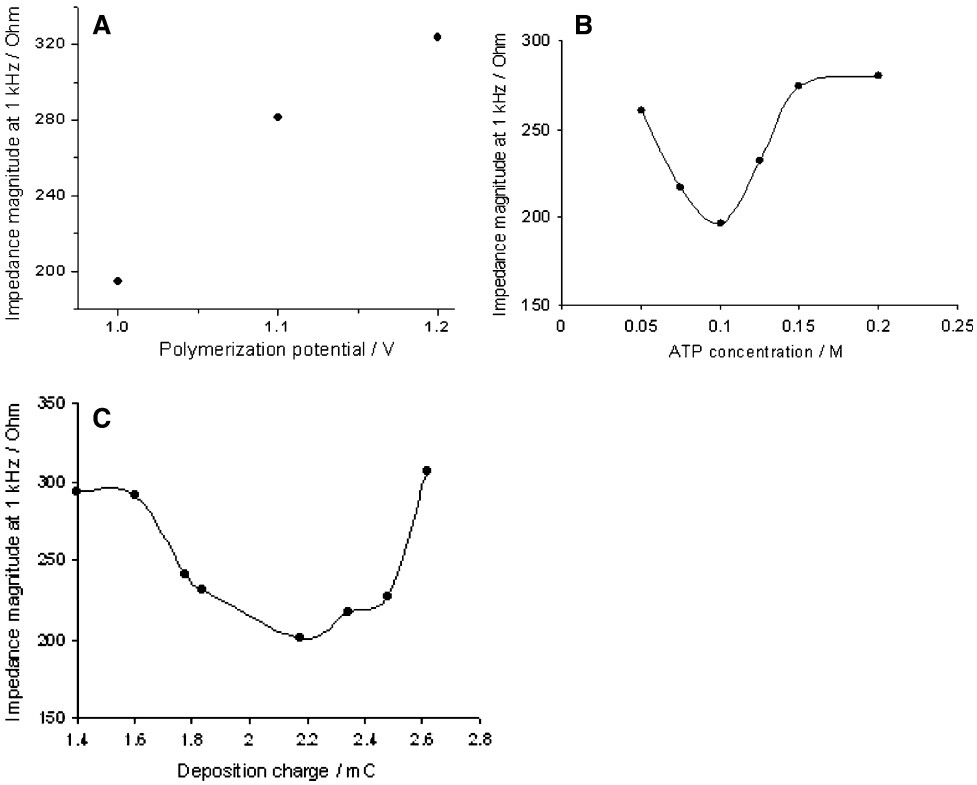


**Fig. 2** Relationship between impedance of the resultant polymer and polymerization potential (**A**), ATP concentration (**B**) and deposition charge (**C**)

saturated by the counterions and the remaining ATP molecules are physically adsorbed on the polymer surface, which blocks the electrode surface and induces an undesired impedance increase.

From the above study we chose 1.0 V as the deposition potential and 0.1 M as the ATP concentration. Electrochemical deposition of PEDOT/ATP was carried out for different time periods. The impedance at 1 kHz also presented a volcanic shape with the passing charges during the deposition (Fig. 2C). The charge quantity of 2.2 mC was the optimum to obtain PEDOT/ATP film with low impedance.

PEDOT/ATP was electrosynthesized for different time, i.e. with different passing charges. AFM images clearly show that the surface roughness varied with time. The AFM results indicate that initially the polymer presents a uniform particle structure (Fig. 3Aa). With time increasing, surface morphology changes from particles to nodules (Fig. 3Ab) and finally to clusters (Fig. 3Ac). The section analyses reveal that the surface roughness increases followed by decrease with the passing charges. The characteristic size of the texturing changes from 50 to 500 nm. The variation of the surface topography is in agreement with the change of impedance, which is also reported previously [17].

Drug release property of ATP from PPy was investigated and reported [24]. In this work stability of the incorporated ATP in PEDOT was investigated by using UV-visible spectrum and impedance spectroscopy. The release behavior of ATP from PEDOT at open circuit was studied over an extended period of time. ATP has a characteristic absorption at 260 nm. The absorbance could be applied to determine the amount of ATP released from PEDOT in the bath solution. From Fig. 4 we observed that the spontaneous ATP release was slightly active within the first two days and the polymer ultimately became stable after 2 weeks. The spontaneous release came from some loosely bound ATP and was rather limited. This indicates that ATP could remain stable after incorporation into the PEDOT matrix. As discussed in Sect. 1, ATP is expected to have affinity to neural cells and increase the biocompatibility of the modified electrode because of its inherent biological properties. Hence the stability of ATP in the polymer film is very important for its implantable application.

Impedance reflects the electrical properties of the electrode materials. Impedance variation was monitored while the coated electrode was immersed in 0.1 M NaCl solution up to 2 weeks as shown in Fig. 5. Randle equivalent circuit has been used to explain the impedance data obtained from conductive polymers such as PPy [25, 26], which is composed of the ohmic resistance of the electrolyte solution, $R_s$, in connection in series with parallel elements of double layer capacitance, $C_{dl}$, and Faraday impedance, $Z_f$. $Z_f$ often comprises serially connected electron-transfer resistance, $R_{et}$, and Warburg impedance, $Z_w$, resulting from the

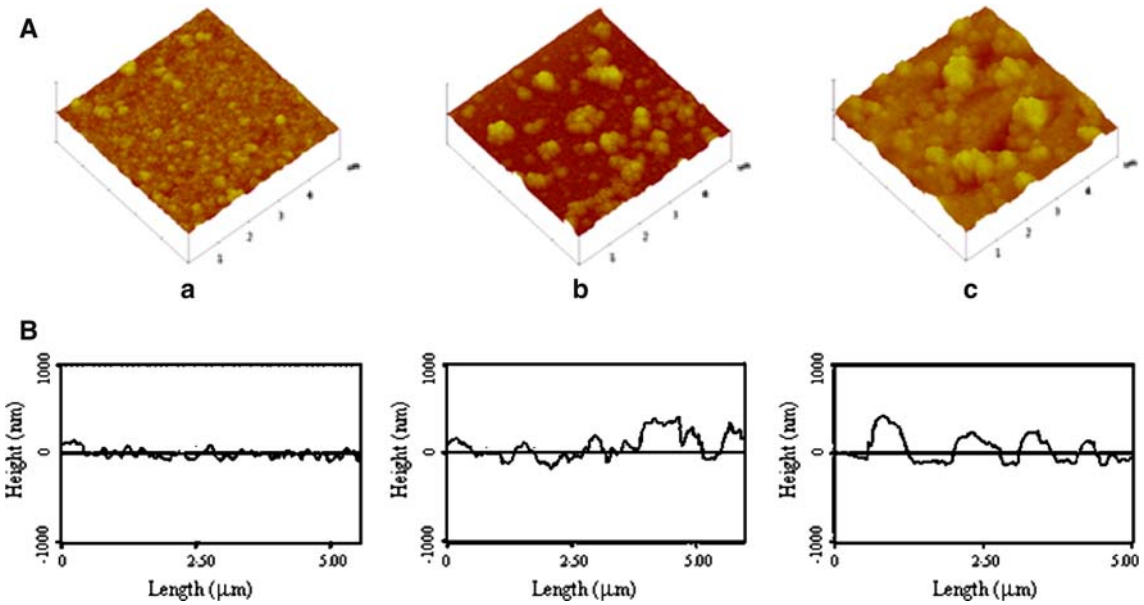


**Fig. 3** AFM images of PEDOT/ATP with different deposition time (**A**) and corresponding section analyses (**B**). Deposition charge was 1.5 mC (**a**), 2.2 mC (**b**) and 4.5 mC (**c**), respectively

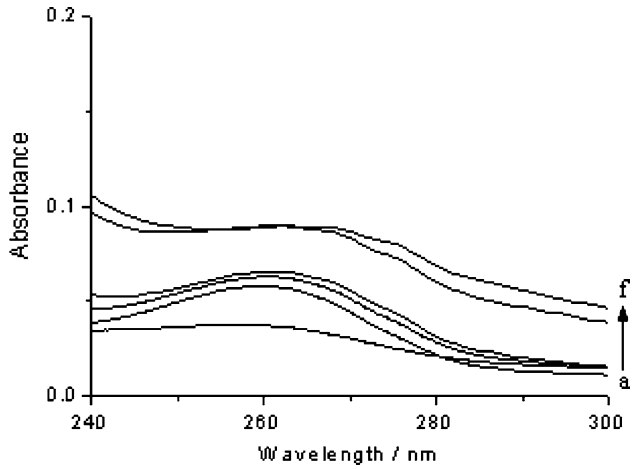


**Fig. 4** UV absorbance of ATP spontaneously released from PEDOT/ATP. Immersion time was (**a**) 1 day, (**b**) 2 days, (**c**) 4 days, (**d**) 7 days, (**e**) 14 days and (**f**) 18 days

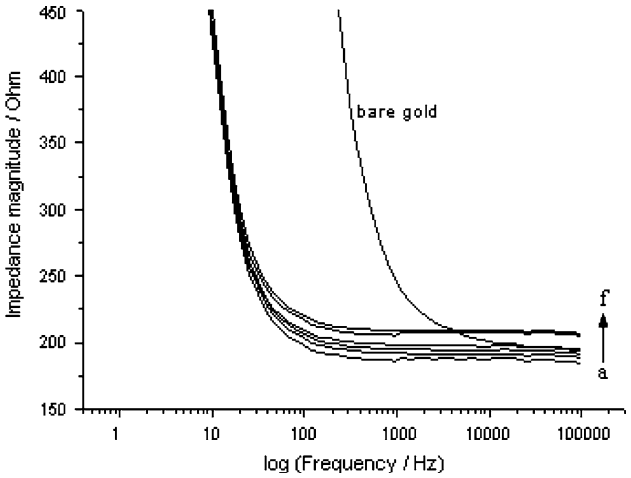


**Fig. 5** Impedance spectra of PEDOT/ATP coated electrode after immersion in NaCl solution for (**a**) 0 day, (**b**) 2 days, (**c**) 4 days, (**d**) 7 days, (**e**) 14 days and (**f**) 18 days

diffusion of ions from the bulk electrolyte to the electrode surface. The impedance magnitude in Fig. 5 equals to the square root of sum of square of imaginary part ($C_{dl}/2\pi f$, $f$ is the frequency) and square of real part ($R_s + Z_f$). That is why the impedance magnitude significantly increases with decrease of the frequency over a low frequency range. However, when the frequency increased to 100 Hz and above, the impedance magnitude is mainly controlled by the real impedance part, DC resistance ($R_s + Z_f$) and becomes flat. As revealed in Fig. 5, before immersion, the impedance of the polymer-coated electrode at frequencies larger than 100 Hz is much smaller than that of the bare gold electrode due to the better apparent conductivity of PEDOT, which in turn is probably due to the higher roughness of the PEDOT modified Au electrode. After submerged in NaCl solution, the impedance increased slightly with submerging time. This is very likely due to its initial instability in the solution [27]. However impedance does not have noticeable change after 14 days, indicating a stability of ATP in the polymer matrix. This good stability can be ascribed to ATP dopant, which is a large counterion and can significantly retard polymer degradation [28]. The observation is in agreement with UV spectroscopy. We draw a conclusion that ATP could be strongly electrostatically bound with PEDOT and the property favors its

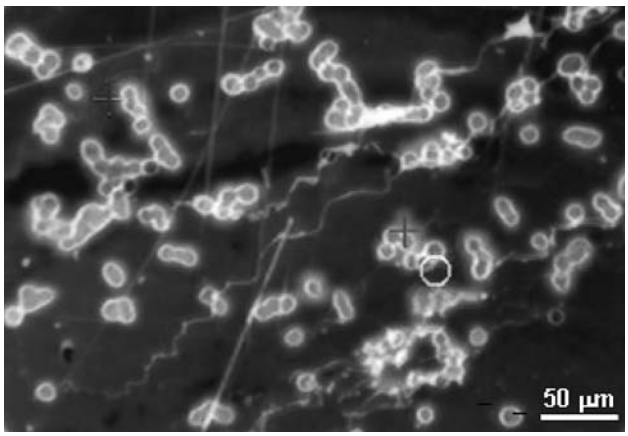


**Fig. 6** Cell adhesion on PEDOT/ATP coated electrode after 4-day culture

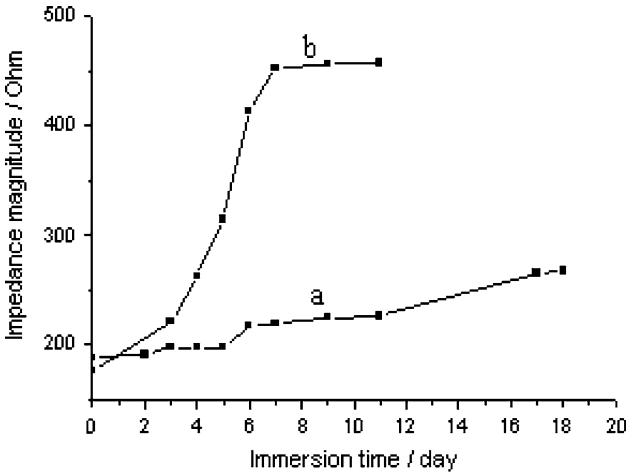


**Fig. 7** Impedance variation of PEDOT (**a**) and PPy (**b**) with immersion time in GSH

application as a substrate for cell attachment due to the cell affinity of ATP.

The PEDOT/ATP and PEDOT/$LiClO_4$ (control for comparison) coated gold electrode were cultured with PC12 cells for 10 h. The time span was long enough for the cells to adhere tightly onto the material surface, i.e. cell adhesion occurred, and was short enough to prevent the possibility of proliferation. We found that after 10 h more cells were adhered on PEDOT/ATP than on PEDOT/$LiClO_4$ (data not shown). Statistical analysis reveals that the adhesion percentage of the former was twice as many as that of the latter. The phenomenon is reasonable because ATP is a kind of biomolecule and thus has affinity to cells.

In order to further investigate the cell growth on the PEDOT/ATP coated electrode, microscopic images were taken after four days. From Fig. 6 we can see that PC12 cells are attached to the polymer surface and sequentially grow. On one hand the polymer has rough morphology thus higher surface area as we reported previously [17]. Such kind of surface would evidently have stronger anchorage to cells. On the other hand, the biomolecule of ATP actually has inherently high bioaffinity and thus plays an important role in the adhesion of neural cells. The results demonstrate that PEDOT/ATP is an excellent substrate for cell adhesion, which can support the survival of PC12. The biocompatibility would facilitate its application as a modifier of implantable device.

After implantation, the polymer modified electrode requires surviving in the physiological fluids and thus the stability of the electrode material against biological reducing agents is very critical for its long-term performance. The regioregular chemical structures in PEDOT are more than those in PPy and are expected to make PEDOT more stable in biological fluids. As it is known the conductive form of a conducting polymer corresponds to its oxidized state. When a conducting polymer coated electrode is immersed in a reducing agent, redox reaction would occur, which causes the polymer to change from its oxidized state to neutral form, resulting in its conductivity loss. GSH is a weak but biological reducing agent and exists both inside and outside cells. When conducting polymers lose their conductivity, impedance will increase. From Fig. 7 we see that the impedance of both PEDOT and PPy increased with immersion time in GSH. This is reasonable due to the redox reaction between the conducting polymers and GSH. However the increasing rate of PPy was much higher than that of PEDOT, indicating that PEDOT is more stable to reduction by GSH than PPy.

The intended application life for the electrodes (neural prosthetic devices) is from weeks to months. It is very difficult to keep an electrode with consistent electrical properties in such a long life. However, an electrode with insignificant or predictable variations is very feasible in practical applications, since calibration in the measurements could be possibly enabled. Apparently, PEDOT/ATP should be a much better candidate than PPy.

## 4 Conclusion

Electrochemical deposition of PEDOT/ATP on gold electrode surface was investigated. Results show that 1.0 V, 0.1 M and 2.2 mC is the optimal deposition potential, dopant concentration and quantity of the passing charges, respectively. After incorporation, ATP inactively and spontaneously releases from PEDOT in the incubation medium and finally remains stable in the polymer matrix after 2 weeks. The stability property is of great importance for PEDOT/ATP to be used as the electrode material for implantable devices in biological environment. The PEDOT/ATP modified electrode exhibited good biocompatibility

with neural cells. PC12 cells preferred to adhere rather to PEDOT/ATP than to PEDOT/$LiClO_4$ within 10 h and subsequently grow on the substrate. Moreover, PEDOT shows excellent stability against biological reducing agent due to its more ordered molecular structure. Our investigation demonstrates that PEDOT/ATP is a promising electrode material to obtain stable and long-lasting neural recording devices.

**Acknowledgment** The work is financially supported by the Center for Advanced Bionanosystem, Nanyang Technological University.

## References

1. Webb K, Budko E, Neuberger TJ, Chen S, Schachner M, Tresco PA (2001) Biomaterials 22:1017–1028
2. Xiao YH, Cui XY, Hancock JM, Bouguettaya M, Reynolds JR, Martin DC (2004) Sens Actuators B 99:437–443
3. Moxon KA, Leiser SC, Gerhardt GA, Barbe KA, Chapin JK (2004) IEEE Trans Biomed Eng 51:647–656
4. Hoogerwerf AC, Wise KD (1994) IEEE Trans Biomed Eng 41:1136–1146
5. Edell DJ, Toi VV, McNeil VM, Clark LD (1992) IEEE Trans Biomed Eng 39:635–643
6. Aylward WM, Pickup PG (2007) Electrochim Acta 52:6275–6281
7. Cui X, Li CM, Zang J, Zhou Q, Gan Y, Bao H, Guo J, Lee VS, Moochhala SM (2007) J Phys Chem C 111:2025–2031
8. Xiao YH, Che JF, Li CM, Sun CQ, Chua YT, Lee VS, Luong JHT (2007) J Biomed Mater Res 80A:925–931
9. Berdichevsky Y, Lo Y-H (2006) Adv Mater 18:122–125
10. Drillet J-F, Dittmeyer R, Juttner K (2007) J Appl Electrochem 37:1219–1226
11. Rumbau V, Pomposo JA, Eleta A, Rodriguez J, Grande H, Mecerreyes D, Ochoteco E (2007) Biomacromolecules 8:315–328
12. Xiao YH, Cui X, Martin DC (2004) J Electroanal Chem 573:43–48
13. Jang J, Chang M, Yoon H (2005) Adv Mater 17:1616–1620
14. Yamato H, Ohwa M, Wernet W (1995) J Electroanal Chem 397:163–170
15. Heywang G, Jonas F (1992) Adv Mater 4:116–118
16. Groenendaal LB, Jonas F, Freitag D, Pielartzik H, Reynolds JR (2000) Adv Mater 12:481–494
17. Xiao YH, Li CM, Yu SC, Zhou Q, Lee VS, Moochhala SM (2007) Talanta 72:532–538
18. Wallace GG, Kane-Maguire LAP (2002) Adv Mater 14:953–960
19. Richardson RT, Thompson B, Moulton S, Newbold C, Lum MG, Cameron A, Wallace GG, Kapsa R, Clark G, O'Leary S (2007) Biomaterials 28:513–523
20. Kamei J, Takahashi Y, Yoshikawa Y, Saitoh A (2005) Eur J Pharm 528:158–161
21. Siow NL, Xie HQ, Choi RCY, Tsima KWK (2005) Chem Biol Interact 157–158:423–426
22. Matsumoto N, Sorimachi M, Akaike N (2004) Brain Res 1009:234–237
23. Sakmeche N, Aeiyach S, Aaron J-J, Jouini M, Lacroix JC, Lacaze P-C (1999) Langmuir 15:2566–2572
24. Pernaut J-M, Reynolds JR (2000) J Phys Chem B 104:4080–4090
25. Li CM, Sun CQ, Song S, Choong VE, Maracas G, Zhang XJ (2005) Front Biosci 10:180–186
26. Li CM, Chen W, Yang X, Sun CQ, Gao C, Zheng ZX, Sawyer J (2005) Front Biosci 10:2518–2526
27. Chen W, Li CM, Chen P, Sun CQ (2006) Electrochim Acta 52:1082–1086
28. Skotheim TA, Reynolds J (1998) Handbook of conducting polymers, 2nd edn. Marcel Dekker, New York