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A Facile Strategy for Two-Step Fabrication of Gold Nanoelectrode for *in Vivo* Dopamine Detection

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Abstract: *In vivo* monitoring neurochemicals with microelectrode is invasive and the damage to brain tissue may inevitably cause disturbance signals physiologically to the measurement. It is of great importance to reduce the electrode size and to decrease the damage. This study demonstrates a novel nanoelectrode preparation methodology for *in vivo* monitoring dopamine (DA) fluctuation in the living brain of rats with high dependability. The fabrication process of the gold nanoelectrode involving a few minutes consists of only two steps: 1) growing gold nanoseeds on surface of tip of glassy capillary by ion sputtering; 2) wet depositing a continuous conductive gold film composed of gold nanoparticles by dipping the capillary with gold nanoseeds into a freshly mixed chloroauric acid and hydroxylamine hydrochloride for one minute. The tip size of the well-prepared gold nanoelectrode was 300 ~ 400 nanometers. The gold nanoelectrode was able to detect DA and showed a good linearity with the concentration of DA ranging from 1.0 to 56.0 μmol·L^{-1} with a limit of detection as low as 0.14 μmol·L^{-1} (S/N=3). Benefiting from the excellent electrochemical performance, the gold nanoelectrode was successfully employed for catecholamine release in striatum of living rat brain.

Key words: ion sputtering; wet deposition; gold nanoelectrode; *in vivo*; dopamine
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In vivo monitoring dynamic changes of neurotransmitters have become more and more crucial to understand and uncover central nervous system behavior and function[1-2]. Microelectrode could track the dynamics of neurochemicals with a high spatiotemporal resolution[3-5]. However, traditional method of microelectrode inserting into brain is invasive, and as a result, it might cause a small damage on brain tissue[6]. Some microelectrodes possess the size of several to hundreds of micrometers. Once the brain tissue was ruined, the obtained neurochemical signals might be disturbed and could not reflect the real physiological and pathological processes.

Reducing size of microelectrode is a candidate to minimize the damage to brain and thus nanoelectrode is of great significance for *in vivo* detection considering minimization the disturbance to brain tissue to the most extent. For instance, Jiang et al. fabricated a microelectrode with a 2 μm tip to detect hydrogen peroxide[7], in which the cell still possessed viability after the electrode insertion since the electrode was small enough. Besides the minimal damages to cell and *in vivo* tissue, the smaller the microelectrode is, the more precisely the microelectrode can be positioned into a narrow biological space. We ever presented the fabrication of addressable carbon-deposited microelectrode arrays with each carbon microelectrode to be 3 ~ 4 μm and observed vesicle release from the single PC 12 cell which could distinguish subcellular spatial difference during the exocytosis process[3]. Li et al. made a nanotip electrode to detect individual vesicles containing catecholamine inside living PC 12[8]. Mirkin's group[9] and Huang's group[10] have established carbon based nanoelectrode loading platinum to quantitatively measure the amounts of active and reactive oxygen/nitrogen species in

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phagolysosomes of single cell. In order to expand the applications of nanoelectrodes in cell and living animals, developing more methods to fabricate nanoelectrode based various materials is necessary. Recently, gold electrodes have attracted increasing interests because of their chemical inertness and resistance in different environmental conditions, high overall conductivity, relatively low-cost and conveniently subsequent modifications[11-12]. Thus, recent developments in gold microelectrode fabrications and surface modifications have been reinvigorated. Developing easy-to-use and high-efficiency methods for fabrication versatile gold electrodes are highly desired[13].

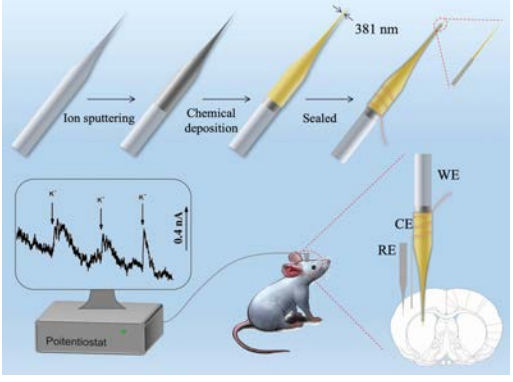
Dopamine (DA) is one of the most considerable catecholamine neurotransmitters, playing a major part in function of central nervous system for instance memory and learning. Disordered levels of DA in the central nervous system have been involved in some neurodegenerative diseases including Alzheimer's diseases Parkinson's disease and schizophrenia[14-17]. Observing the real-time kinetics change of DA *in situ* is paramount to uncovering these functionalities and extending our understanding of brain function and dysfunction. In our early study, we found that the gold based electrode exhibited a special long-term stability at the time of detecting DA against chemical fouling higher than carbon based electrodes[18]. In addition, Zhu et al. designed a glass-sealed gold nanoelectrode for cerebral DA detection[12]. Lately, we reported a facile wet deposition technique and a novel ultrasonic-aided process to build nanostructured gold-ring microelectrodes for electrochemical monitoring of catecholamine release from rats brain and cells[13, 18]. However, the problems in these progresses are either that sophisticated instrument of laser puller is essential or that it is not suitable for preparing nanoelectrode, since the fragile nanotip capillary would be broken during the ultrasonication with high energy.

The previous study provides a new method to fabricate a gold nanoelectrode with high reliability for application of *in vivo* detection of catecholamine in the living brain. Ion sputtering is a common strategy to coating gold or platinum nanoparticles on surface of substrate and to enhance the conductivity of samples[18] which can be utilized to sputtering gold nanoparticles on surface of capillary nanotip. As shown in Scheme 1, this method only contains two steps, growing gold nanoseeds on tip surface of glassy capillary by ion sputtering, and followed by wet depositing a continuous conductive gold film on the surface of capillary. This strategy avoids hazardous chemical solution piranha solution to hydroxylate surface of glass. In addition, the mild condition without assistance of ultrasonic wave endows the formation of a well conductive gold film from the nanotip to the whole capillary. The fabrication process of the gold nanoelectrode involved a few minutes and the well prepared gold nanoelectrode was successfully employed for catecholamine release in striatum of living rat brain during stimulation of high concentration of K^+.

1 Experimental

1.1 Materials

Potassium nitrate (KNO_3), monopotassium phosphate (NaH_2PO_2), sodium hydroxide (NaOH), L-cysteine, glucose, potassium chloride (KCl), hydrochloric acid (HCl), sodium chloride (NaCl), ethanol (C_2H_5OH) and transparent nail enamel were purchased from Beijing Chemical Reagent Company. Dopamine hydrochloride (DA), chloroauric acid tetrahydrate ($HAuCl_4 \cdot 4H_2O$), magnesium chloride ($MgCl_2$),



Scheme 1. Schematic illustration showing the fabrication progress of gold nanoelectrode and monitoring DA fluctuation in striatum of rat brain.

calcium chloride ($CaCl_2$), ferrocenemethanol ($FcCH_2OH$), hydroxylamine hydrochloride ($NH_2OH \cdot HCl$), HEPES, platinum wire and silver wire were obtained from Sigma-Aldrich. All reagents were used without further purification. The high concentration of K^+ solution consisted of KCl (70 mmol·L^{-1}), NaCl (78 mmol·L^{-1}), glucose (10 mmol·L^{-1}), $CaCl_2$ (2 mmol·L^{-1}), $MgCl_2$ (1 mmol·L^{-1}), HEPES (10 mmol·L^{-1}) and Milli-Q water, and pH was adjusted to 7.4[19]. All aqueous solutions were prepared using double distilled water made by a Milli-Q instrument. Glass capillaries (diameter of 0.86 mm inside and 1.5 mm outside,) were purchased from Sutter Instrument Company of USA.

1.2 Preparation of Nanoelectrodes

Glass capillaries with tips to be about 100 nm were pulled by an apparatus of Model P-97 puller (Sutter Instrument Company, USA) with the parameters of HEAT 560, PULL 55, VEL 95, TIME 200, P 200. The gold seeds were coated at surfaces of pulled capillaries by E-1045 Ion Sputter Instrument (Hitachi, Japan) for 1 minute. Subsequently, the capillaries coated with gold seeds were immersed in a freshly mixed solution of 0.1wt% $HAuCl_4 \cdot 4H_2O$ and 0.04 mol·L^{-1} $NH_2OH \cdot HCl$ with volume ratio of 10:1 for 1 minute, in which $HAuCl_4$ acted as an oxidant and $NH_2OH \cdot HCl$ played a role of reductant. In this solution, a lot of gold nanoparticles were grown from the gold nanoseeds and finally forming the conductive gold film. Next, copper wire was twined on gold film. Finally, nail enamel was used to seal the as-prepared electrode remaining 1 ~ 2 mm to detect DA *in vivo*.

1.3 Instruments and Apparatus

All electrochemical tests were performed on an electrochemical workstation (CHI 1030C, Shanghai, China). Three-electrode system was applied with the as-prepared gold nanoelecrtrode as the working electrode, Ag/AgCl wire as the reference electrode and platinum wire as the counter electrode. In the *in vivo* experiment, in order to reduce the sizes of counter electrode and reference electrode to the most extent, a 100 μm diameter platinum wire was used as the counter electrode and the micro reference electrode was prepared by depositing AgCl on a 100 μm diameter silver wire. During the preparation of nanoelectrodes, an optical microscope equipped with scale (IX73, Olympus, Japan) was used to observe tips of capillaries. Field emission scanning electron microscopic (FE-SEM) images were obtained by a Hitachi SU-8010 microscope.

1.4 Animal Experiments

Adult male Sprague-Dawley (SD) rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., weighing 300 ± 50 g. The SD rats were entertainment on a 12:12 h light-dark schedule with water and food ad libitum. Animal experiments were performed during the light cycle as convention. Briefly, after being anesthetized with 10wt% chloral hydrate via intraperitoneal injection (345 mg·kg^{-1}, ip), the rats were placed on a stereotaxic frame with the incisor bar located 5 mm above the interaural line. The skulls of rats were drilled suitable holes through for inserting electrodes. The gold nanoelectrode was implanted into striatum (L = 3 mm from bregma, AP = 0 mm, V = 4.5 mm from dura) through a standard stereo positioning process monitoring the levels of DA. Infusion solutions were delivered by medical micro injection syringe. Silver wire was polarized at constant 0.6 V in 0.1 mol·L^{-1} HCl for 30 minutes to fabricate micro reference electrode[20]. The prepared Ag/AgCl microsized reference electrode was located in the dura of brain. Stainless platinum wire was employed as the counter electrode by implanting into subcutaneous tissue on the brain. Additional chloral hydrates were injected if it has been a long time during the surgery, and a heating panel was used to maintain the body temperature of the rats at 37 °C. Constant potential of 0.3 V versus Ag/AgCl was employed to the electrochemical recording channel for *in vivo* measurements of catecholamine in living rat brain.

2 Results and Discussion

2.1 FE-SEM Images of Gold Nanoelectrodes

To characterize the morphology of gold nanoseeds on tip of capillary and the finally well prepared nanoelectrodes, SEM was performed carefully. Fig. 1A

displays the SEM image and optical image of freshly pulled glassy capillary, which is smooth and transparent. Compared with the pure capillary, SEM image and optical image of ion sputtering treated capillary (Fig. 1B) clearly shows the presence of gold nanoseeds on the capillary and capillary color turning to light gray, which demonstrates that gold nanoseeds were sputtered on glassy capillary successfully. After the wet chemical deposition, Fig. 1C and Fig. 1D show morphologies of the as-prepared nanoelectrodes with gold nanoparticles in random orientations, which validate that gold nanoparticles were generated on surface of capillary by wet chemical deposition process. The complete cover of gold nanoparticles on the tip of the nanoelectrode may benefit the good conductivity and the later electrochemical measurements. Compared with the bare capillary with no gold seeds, the gold nanoparticles could hardly deposit on the surface of capillary (data not shown). We speculate that this phenomenon might be due to tight gold seeds that could induce the formation of gold nanoparticles. The tip of well prepared nanoelectrode is approximately 380 nm, which could be used as a nanoelectrode for *in vivo* analysis.

Furthermore, the influence of wet chemical deposition time on size of electrode tip was studied. The gold seeds deposited capillaries were dipped in freshly mixed $HAuCl_4 \cdot 4H_2O$ and $NH_2OH \cdot HCl$ solution for various time, including 1 min, 2 min, 5 min and 10 min, and the corresponding SEM images are illustrated in Fig. 2A-D, in which the resultant diameters of each capillary tip are about 381 nm, 1.58 μm, 2.59 μm and 5.73 μm, respectively. The present findings revealed that the size of AuNPs coated capillary tip was increased with the wet increasing of chemical deposition time.

2.2 Electrochemical Characterization and the DA Sensing of the Fabricated Gold Nanoelectrode

To investigate the gold film successfully formed on the electrode, the gold nanoelectrode was coupled with a platinum wire as the counter electrode and a micro Ag/AgCl wire as the reference electrode, while saturated KNO_3 salt bridge was used to avoid inducing chloride ion into the testing solution. The cyclic voltammogram of gold nanoelectrode in 0.5 $mol \cdot L^{-1}$

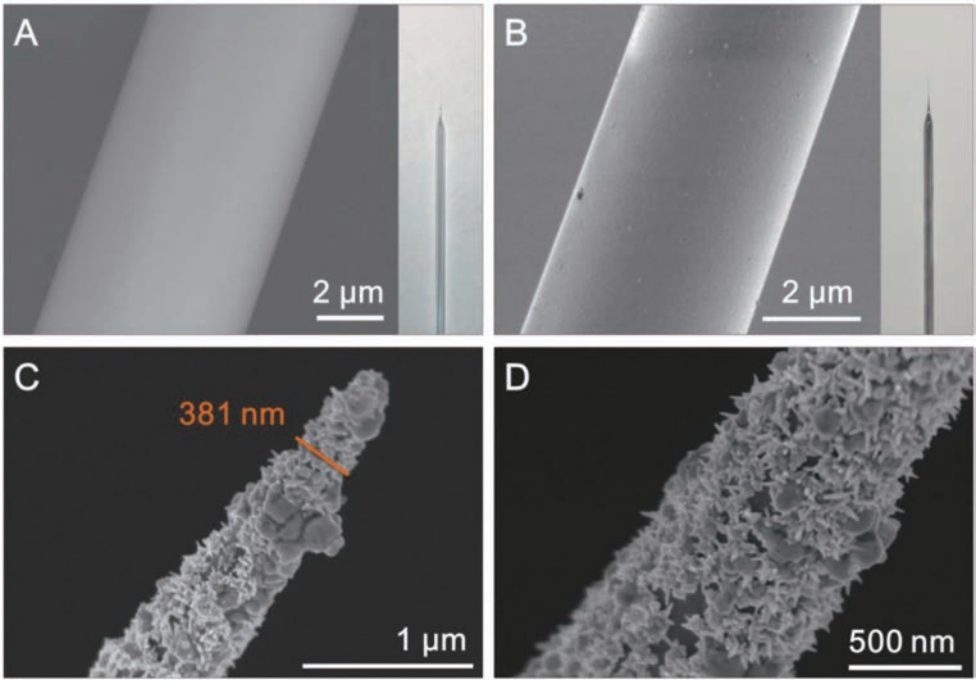


Fig. 1 SEM images and optical images of capillary (A) and capillary with gold nanoseeds (B). (C, D) Capillaries coated with gold nanopaticles after wet chemical deposition treatment.

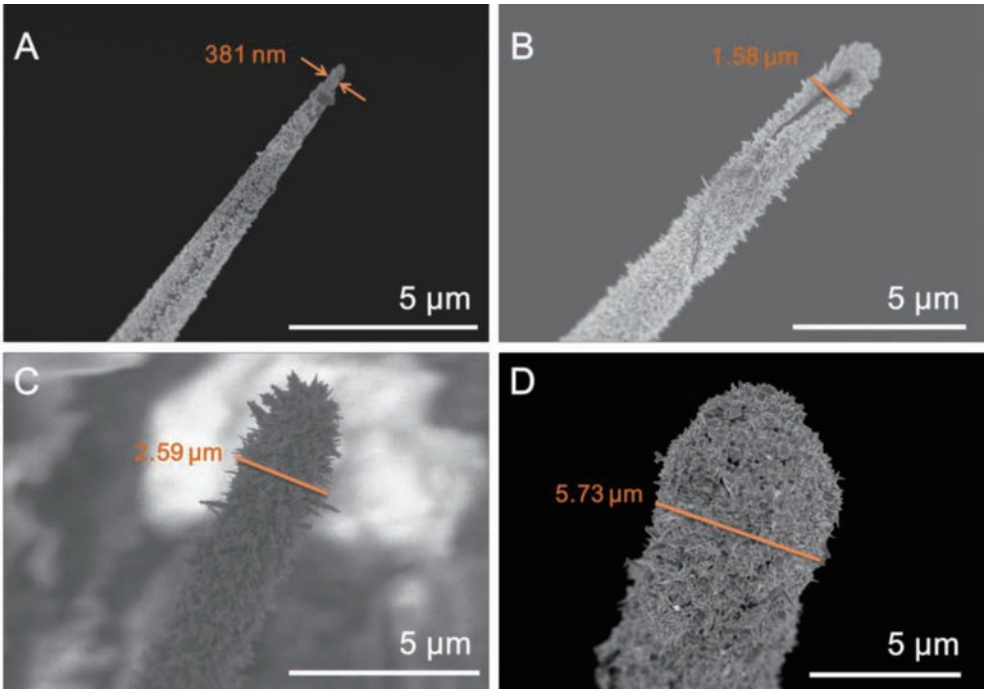


Fig. 2 SEM images of microelectrodes with various time of wet chemical deposition. (A) 1min; (B) 2 min; (C) 5 min; (D) 10 min.

H_2SO_4 is shown in Fig. 3A. There were not only three typical oxidation peaks at 1.195 V, 1.392 V and 1.6 V, but also one obvious reduction peak at around 0.9 V, confirming that the gold film was successfully formed on the capillary tip[18, 21-22]. Furthermore, no apparent changes in CV curves were observed by repeating for 3 cycles, demonstrating the high stability of the gold nanoelectrode in H_2SO_4. In addition, as shown in Fig. 3B, the steady-state electrochemical behavior of the nanoelectrode was tested in 0.2 mol·L^{-1} KNO_3 containing 1 mmol·L^{-1} $FcCH_2OH$. Along the scan rates increasing from 10 to 500 mV·s^{-1}, the CV curves were kinetically quasi-reversible and appeared the as expected typical sigmoidal shape at a low scan rate, demonstrating radial-type and fast diffusion of $FcCH_2OH$ on the electrode. Furthermore, the cyclic voltammograms confirmed that the limiting currents were mostly independent of scan rate, indicating a kinetically quasi-reversible process.

Before *in vivo* monitoring DA, *in vitro* experiments were performed, and the results are shown in Fig. 4. Cycle voltammograms of gold nanoelectrode

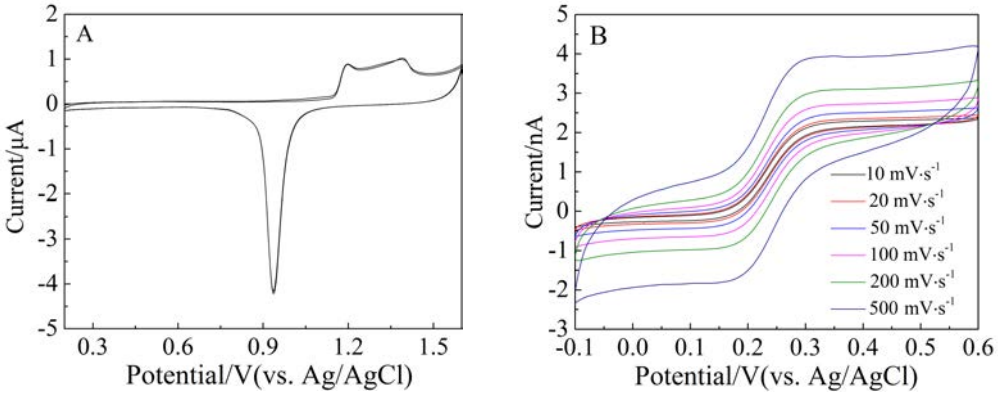


Fig. 3 (A) Cyclic voltammetric curve of gold nanoelectrode in 0.5 mol·L^{-1} H_2SO_4. (B) Steady-state voltammetric responses of the gold nanoelectrode at various scan rates in 0.2 mol·L^{-1} KNO_3 containing 1 mmol·L^{-1} $FcCH_2OH$.

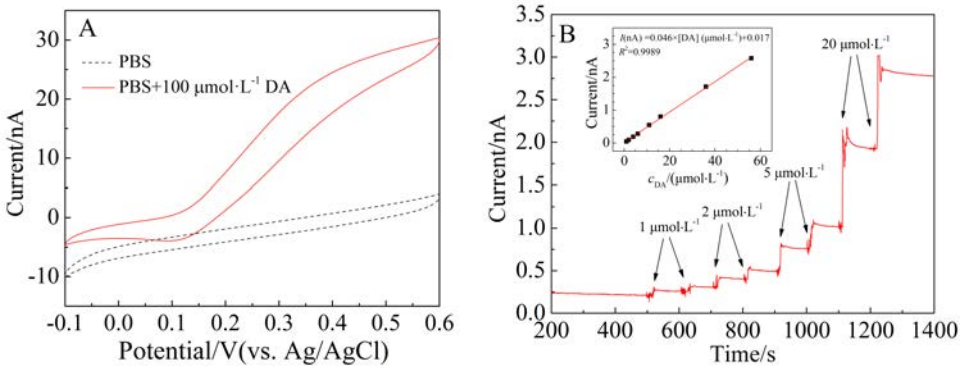


Fig. 4 Cycle voltammetric responses of gold nanoelectrode on detection of DA in PBS (pH=7.4) (black line) and 100 μmol·L^{-1} DA solution (red line). (B) Amperometric *I-t* curve of gold nanoelectrode for the sequential addition of DA measured at 0.3 V (vs. Ag/AgCl). Inset: Fitting plot of DA current versus concentration.

show that there were no redox peaks in PBS, while in the presence of 100 μmol·L^{-1} DA, the oxidation of DA at the gold nanoelectrode occurred at around 0.15 V with the overall sigmoidal shape DA voltammetric behavior, illustrating a fast radial-type diffusion model to the electrode[16,22]. Based on the cyclic voltammograms, the amperometric *I-t* curves of gold nanoelectrode for the sequential addition of DA were measured with the potential poised at 0.3 V in Fig. 4B. The steady-state currents increased proportionally with increasing DA concentrations ranging from 1.0 to 56.0 μmol·L^{-1} (I(nA)= 0.046×[DA](μmol·L^{-1}) + 0.017) with a correlation coefficient of 0.9989, and the detection limit was calculated to be as low as 0.14 μmol·L^{-1} (S/N=3) for DA detection in vitro. Thus, the gold microelectrode could act as an electrochemical sensor for the detection of DA.

2.3 *In Vivo* Amperometric Detection of Catecholamine Release from Rat Brain

To detect DA and other catecholamine *in vivo*, the selectivity of the gold nanoelectrode is important considering many electroactive species coexisting with DA in rat brain. For instance, ascorbic acid (AA) with much higher concentration than DA might provide a main interference encountered in the measurement of DA[23]. Previous study reported that the L-cysteine self-assembled gold electrode could resist the interference in the presence of AA since the electronegative carboxylic group could selectively exclude the negative AA and attract the positive DA[24]. Thus, before *in vivo* assay, our gold nanoelectrode was dipped in N_2-saturated 1 mmol·L^{-1} L-cysteine solution for 1 h. The L-cysteine modified gold nanoelectrode was poised at 0.3 V to *in vivo* detect the evoked catecholamine release, with an Ag/AgCl wire reference and a platinum wire counter electrode. Fig. 5 shows the typical current response acquired by the gold nanoelectrode in the left striatum of living brain during high concentration K^+ injection. As can be seen, the current appeared three distinct increases while high concentration K^+ was injected into the rat brain at 938 s, 1100 s and 1248 s. Specifically, neurons containing a lot of vesicles would release catecholamine neurochemicals with simulation of high concentration K^+[25-27]. Generally, the current responses

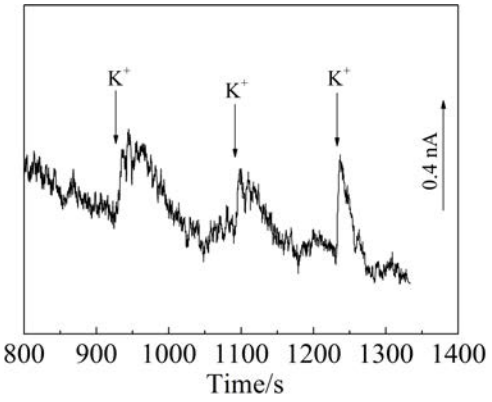


Fig. 5 The L-cysteine modified gold nanoelectrode recorrding catecholamine release from rat striatum evoked by high concentration K^+ at 0.3 V (vs. Ag/AgCl). The stimulation was implemented at 938 s, 1100 s and 1248 s.

should become lower with the increasing of injection time, which agreed well with the previous report[22]. But the third current response was larger than the formers, which might be attributed to that the volume of the third injection of stimulated K^+ was more than the former stimulation. Overall, these results indicated that the fabricated gold nanoelectrode in this study could be used for reliable *in vivo* electrochemical neurotransmitters detection.

3 Conclusions

A new method to prepare gold nanoelectrode based on growing gold nanoseeds was developed by ion sputtering as the initiator material to induce sequent gold nanoparticles-composed gold film grown on capillary. Compared with the existing approaches to fabricate nanoelectrodes, this approach had the advantages in terms of involving a few minutes, convenience and avoiding hazardous chemicals. The gold nanoelectrode exhibited perfect electrochemical property to detect DA *in vitro*. The L-cysteine modified gold nanoelectrode with the improved selectivity to DA was useful for reliable measurement of DA in complex biological system. Accordingly, an *in vivo* electrochemical method has been successfully developed for real-time monitoring of DA in the living rat brain. This study not only prepared a gold nanoelectrode feasible for supervising DA release, but also provided a methodology to fabricate other nanosized metal electrodes for in vivo analysis application.

Acknowledgements

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快捷两步法制备金纳米电极用于活体多巴胺检测

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摘要:用微电极进行活体检测神经化学物质属于侵入式分析,会对脑组织产生不可避免的损伤,进而在生理上产生一些信号干扰检测过程. 减小电极的尺寸对于减小对脑组织的损伤非常重要. 该研究报道了一种新型制备金纳米电极的方法并将其用于活体鼠脑内多巴胺分析研究. 这种金纳米电极的制备过程包含两步:1) 通过离子溅射在毛细管的尖端覆盖一层金种子;2) 把覆盖有金种子的毛细管浸入氯金酸和盐酸羟胺混合溶液中湿法沉积生成连续导电金膜. 制备好的纳米电极尖端约 300 ~ 400 nm. 该金纳米电极可以应用于多巴胺的检测,并且在多巴胺浓度 1.0 ~ 56.0 μmol·L^{-1} 范围内有很好的线性响应,最低检测限低至 0.14 μmol·L^{-1}(信噪比 =3). 该金纳米电极具有优异的电化学性能,可以成功的应用于检测鼠脑纹状体儿茶酚胺的释放.

关键词:离子溅射;化学沉积;金纳米电极;活体分析;多巴胺