An Amplification Strategy to Label Free Opiate Drug Detection using Liquid-Gated Carbon Nanotubes Transistor

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ABSTRACT

The proliferation of narcotic analgesic drug, heroin, has necessitated the development for on-site drug diagnosis. Carbon nanotubes (CNTs), which are renowned for its good biocompatibility and excellent electrical sensitivity, has been an area of active research in the past decade for its applications in biosensing. In this paper, a CNT based field effect transistor (CNTFET) was employed for the direct electrical detection of 6-monoacetylmoprhine. An indirect detection scheme was adopted to enable the detection of morphine antibodies. By incorporating gold nanoparticles to the morphine antibodies, an improvement of the detection limit by two orders of magnitude from 130 fg/ml to ~1 fg/ml was achieved. The mechanism of signal enhancement effect was attributed to the electrostatic gating contribution from the negatively charged gold nanoparticle in addition to the antibodies themselves.

Keywords: carbon nanotube biosensor, carbon nanotube transistor, opiate, real time detection, signal amplification, label-free

1 INTRODUCTION

The potential of carbon nanotube (CNTs) in biosensing application stems from their unique inherent properties such as small size, high strength, high electrical and thermal conductivity, high specific surface area, and good biocompatibility [1, 2]. The extremely high surface-to-volume ratio results in most of the constituent atoms residing on the surface. Thus, binding of biomolecules to the nanotubes leads to the depletion or accumulation of charge carriers throughout the entire nanostructure, making even the slightest disturbance from their proxy surrounding sufficient to alter their electrical properties; an advantage over the conventional planar sensor device [3].

Among the various device configurations and detection methodologies incorporating CNTs in their sensing platforms, liquid-gated field effect transistors (LG-FETs) based architectures outperform the conventional electrochemical measurements by their ability in providing label-free, direct electronic read-out and real-time detection. To date, the sensing capability of CNTFETs has been demonstrated on a variety of molecules from different applications [4, 5], and has been envisaged to complement the current golden standard analytical methods such as ELISA for laboratory uses, point-of-care diagnostic and on-site sample analysis tool.

In this paper, a signal amplification scheme for opiate drug detection was reported using the LG-CNTFET platform. A heroin metabolite, 6-monoacetylmorphine (MAM), was used in the study. Heroin is a naturally occurring substance extracted from unripe seeds or capsules of *Papaver somniferum* (poppy plant) [6]. Upon ingestion, heroin is metabolized sequentially to MAM, morphine, and morphine glucuronide [7]. These drug molecules are potent narcotic analgesics and are often abused as recreational drugs. There is an urgent need to develop a simple and sensitive method to monitor these opiate drugs. As of today, the typical tests to detect these opiate include: electrochemical methods, high-pressure liquid chromatography, mass spectroscopy, and fluorescence immunoassays [6]. Sensitive as they may be, these methods are usually time-consuming, expensive and not adoptable for on-site analysis. Very often, an electrochemical or magnetic tag is required to perform the measurement, inflating the number of the process steps.

Using our previously developed, novel PDMS based, all CNTs LG-FET [8], we have demonstrated the detection of small morphine metabolite through indirect immunoassay approach. The device fabrication offers advantages such as scalability and simplicity, and obviates the requirement for clean room facility, therefore can be applied for disposable, on-site screening purpose.

2 EXPERIMENTAL PROCEDURES

Carboxylated single-walled CNT powder was purchased from Cheaptubes. Inc. PDMS (Sylgard 184) was purchased from, Dow Corning, Inc. 6-Monoacetylmorphine (MAM) was purchased from Cerilliant Analytical Reference Standards Sodium dodecyl sulfate (SDS), tween-20, poly

(ethylene glycol) (PEG), skim milk, bovine serum albumin (BSA), Complete Freund's adjuvant (CFA), Incomplete Freund's adjuvant (IFA), sodium dihydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Sigma Aldrich. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and N-hydroxysulfosuccinimide (sulfo-NHS) were purchased from Pierce Chemicals. Protein-A Sepharose, were procured from Amersham Biosciences, India.

2.1 Biomolecules synthesis

Synthesis of MAM-BSA conjugates

Synthesis of hapten, derivatization was done by refluxing the reaction mixture for 3 h at 90 °C in an inert nitrogen atmosphere, containing 3 μM of MAM, 24 μM of chloroacetic acid, 45 μM sodium hydroxide and 30 μM acetonitrile. The presence of the –COOH group was confirmed by a thin layer chromatograph (TLC) and infra-red (IR) spectroscopy [6]. The derivatized hapten (MAM-COOH) was used for the conjugation with BSA (carrier protein) using carbodiimide coupling chemistry. For the activation, 50 μM MAM–COOH, 75 μM EDC and 75 μM sulfo-NHS were mixed and incubated for 1 h at room temperature, followed by overnight incubation at 4 °C, and centrifuged for 10 min at 10,000×g to remove the urea precipitate. For the conjugation of activated hapten with BSA, 30 μM of activated hapten was mixed with 0.15 μM (10 mg) of BSA to prepare the molar ratio of 100:1.

Generation of morphine antibodies (Mor-Ab)

The morphine antibodies (Mor-Abs) were raised against MAM-BSA conjugate in young six to eight weeks old New Zealand white rabbits. The rabbits were immunized subcutaneously with 250 μg of MAM-BSA mixed with equal volume of Freund's complete adjuvant at the time of the first booster followed by Freund's incomplete adjuvant in subsequent booster doses. The rabbits were bled after 5th day of each booster and blood was collected, serum precipitated and antibodies (IgG) were purified using a Protein A sepharose column. The fractions were then dialyzed against PBS and the IgG concentration was determined at 280 nm and stored at -20°C until used.

Synthesis of gold labled Mor-Ab (Au-Mor-Ab)

Monodispersed (30 nm) colloidal gold was prepared by a modified Frens method. A 200 mL solution of 0.01% tetrachloroauric acid in Milli-Q water was brought to boiling. 4 mL sodium citrate solution (1% w/v) was added to the boiling gold chloride solution. The solution was allowed to boil for 10 min until it developed the typical bright wine red color of colloidal gold. The average particles size of colloidal gold was determined using a transmission electron microscope (Hitachi Model H-7500) operated at 120 kV. The average particle size was estimated to be approximately 30 ± 4 nm.

For the preparation of the antibody gold conjugate, 90 μg of Mor-Ab was prepared in a 20 mM phosphate buffer, pH 7.4 and added drop-by-drop into 1 ml colloidal gold solution ([Au] = $2.4x10^{-4}$ mol/L) under mild stirring condition. The pH of the colloidal gold solution was maintained at 7.4 by addition of 10 mM Na_2CO_3 before adding the antibody. The mixture was incubated overnight at 4°C and centrifuged at 12000 rpm for 30 min to remove the unconjugated antibodies from the solution. The pellet obtained was washed three times with 10 mM Tris (pH 8.0) containing 3% BSA under centrifugation at 12000 rpm for 30 min to remove traces of unconjugated antibodies. The pellet was resuspended in 2 ml of phosphate buffer (20 mM, pH 7.4) and stored at 4°C before its use. The final concentration of colloidal gold in the antibody-gold conjugate solution was $4.8x10^{-4}$ mol/L. A Hitachi 2800 UV-vis spectrophotometer was used to measure the absorbance of gold nanoparticles and Au-Mor-Ab.

2.2 Immunosensor preparation

The fabrication process of flexible, all CNT/PDMS LGFET was described in details in the previous publication [9]. Briefly, only two materials are required in this fabrication route: SWCNT and PDMS. A thin SWCNT random network, formed from suspension through vacuum filtration method [10], was transfer-printed from an alumina filter onto a blank PDMS substrate to form the semiconducting layer, while another thick, metallic-like SWCNT random network prepared from the same methodology was transfer printed onto another PDMS substrate (with an integrated microfluidic channel) as the electrode material. The stamping of CNT on the PDMS with the defined microchannel results in the auto-separation and formation of source-drain pads on each side of the microchannel. Lamination of the two PDMS substrates face-to-face encloses the microfluidic channel and completes the fabrication process.

After the completion of device fabrication, the carboxylic functional groups on the SWCNT at the active channel region were activated with EDC and sulfo-NHS for 1 hour at room temperature, rinsed with 50 mM PB solution (pH 9.5), followed by 10 μg/ml MAM-BSA injection and overnight incubation at 4°C. Excess unbound molecules were removed by rinsing the microchannel with copious of PB solution, and the device was ready for target antibodies detection.

2.3 Electrical measurement

Electrical measurement of the CNT-LGFET was performed using a home-built LabView system with the testing protocol similar to the reported literature [11, 12]. For real time monitoring, a liquid gate potential (V_G) at -0.5 V was applied to the electrolyte through reference electrode (3M KCl, FLEXREF, World Precision Instruments) and a small drain bias (V_D) of 10 mV applied over the source and

drain electrodes to obtain the kinetic response at respective sensing steps.

3 RESULTS & DISCUSSION

The simplest method to detect the MAM or MAM-BSA would be a direct immunoassay: using Mor-Ab as a probe molecule immobilized onto the CNT network (see Fig. 1(i)). However, a charge screening phenomenon by the ions present in the electrolyte diminishes the electrical signal originated from the biomolecular interaction to be detected by the underlying CNT network.

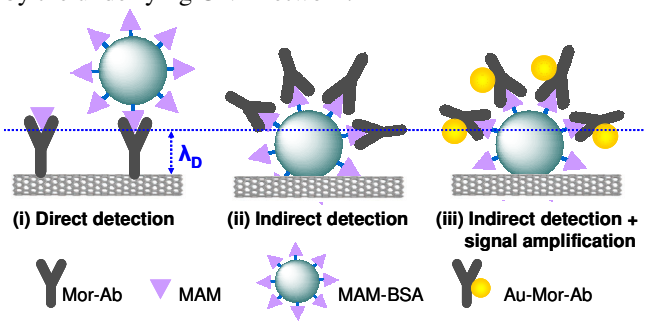

Figure 1: Illustration showing the configurations for different detection schemes.

The length which characterizes this screening effect is represented by the Debye length (λ_D) (Equation (1)). In this equation, the term I represents the ionic strength (mol/m^3), ε_0 is the permittivity of free space, ε_r is the dielectric constant, k represents the Boltzmann's constant, T is the absolute temperature in Kelvin, N_A is Avogadro's number and e is the elementary charge. The inverse relationship between λ_D and the I highlights the importance of ionic-strength of the electrolyte in modulating charge screening effect and hence influences the sensitivity in LGFET.

$$\lambda_D = \sqrt{\frac{\varepsilon_r \varepsilon_0 kT}{2z^2 e^2 I}} \qquad (1)$$

For a 50 mM PB solution used in this study, the λ_D was estimated to be ~3.5 nm. Given the usual size of an antibody (10-15 nm) [13], the immunocomplex formation in a direct immunoassay approach is certainly out of the λ_D distance. One simple alternative is to reduce the concentration of the electrolyte. However, it comes at the expense of reduced binding efficiency. Therefore, an indirect immunoassay approach (Fig. 1(ii)) is proposed wherein the MAM-BSA is used as the receptor for antibody detection. The hapten-carrier conjugation is engaged so that the amine-rich BSA could be bound covalently to the carboxylic groups on the CNT through carbodiimide coupling chemistry, while the MAMs are conjugated to BSA through established protocol to preserve its activity towards the corresponding antibody [6].

The indirect detection scheme allows a fraction of immunocomplexes to fall within the λ_D distance, hence increases the signal level for Mor-Ab detection, as illustrated in Fig. 1(ii). Fig 2 shows the real time data for Mor-Ab detection in a logarithmic serial dilution manner from 10 fg/ml, 100 fg/ml, 1 pg/ml, 100 pg/ml, 1 ng/ml, 10 ng/ml, 100 ng/ml, 1 µg/ml to 10 µg/ml (labeled from 1 to 9 respectively), along with the corresponding calibration plot which is based on triplicate kinetic measurements. The electrical current increment upon the immunocomplex formation was attributed to the electrostatic gating mechanism, where the negatively charged Mor-Ab induces positive doping in the CNTs, raising the overall hole carrier concentration in the p-type CNT transistor, and leading to the increment of overall conductance [8].

To determine the limit of detection (LOD), the calibration plot in Fig. 2(b) was first fitted with a log-linear regression equation at the linear regime from 10 fg/ml to 100 ng/ml. The fitting yields a R^2 of 0.981, indicating good fit with the underlying experimental data. By using Equation (2) and taking the 3σ criterion [14], the LOD for Mor-Ab was estimated to be 130 fg/ml. The term σ in the equation is the standard deviation of the fitted line with the underlying data point; S is the sensitivity calculated from the slope of the linear regime; and C is the curve fitting constant.

$$LOD = (3\sigma / S) + C \qquad (2)$$

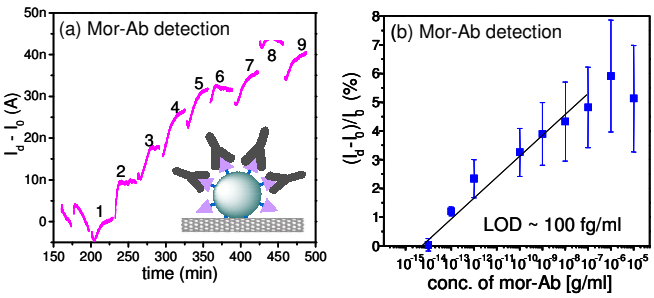

Figure 2: Indirect detection scheme where MAM-BSA is used as the receptor for Mor-Ab detection. (a) Kinetic measurement plot (b) Concentration plot

To further enhance the signal, an amplification strategy was employed by tagging the gold nanoparticles (AuNPs) to the Mor-Ab. AuNPs have been widely used in electrochemical based biosensing as labels for molecule detection, carriers for other electroactive labels, or signal enhancement tools [15]. It was chosen in our study because of its simple geometry and uniform charge distribution. AuNPs typically carry a negative charge as a result of the citrate ion absorption during the nanoparticles synthesis through the citrate reduction method [15]. From the LG-FET perspective, if the sensing mechanism is dominated by electrostatic gating, the addition of AuNPs to the existing detection system should further induce more positive charge carriers into the channel, resulting in larger signal

increment. We verified this alternative route of signal enhancement through an offline experiment to confirm effect of direct AuNPs interaction with CNT network (Fig. 3). Within the pH window tested, increased conductance signal were observed upon the injection of 50 nM AuNPs solution into the microchannel.

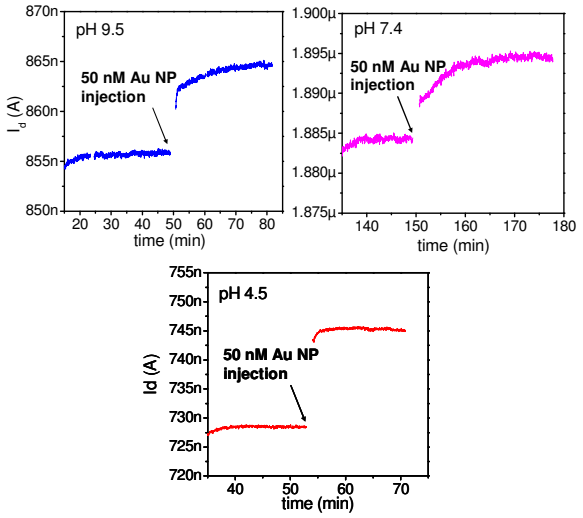

Figure 3: Direct detection of AuNPs on CNT [16].

Fig.4 shows the kinetic measurement with different Au-Mor-Ab concentrations: (1)1 fg/ml, (2)10 fg/ml, (3)100 fg/ml, (4)1 pg/ml, (5)100 pg/ml, (6)1 ng/ml, (7)10 ng/ml, (8) 100 ng/ml,(9) 1 µg/ml and (10) 10 µg/ml, and the corresponding calibration plot based on triplicate kinetic measurements. The estimated LOD was about 1 fg/ml, two orders of magnitude more sensitive than the previous scheme.

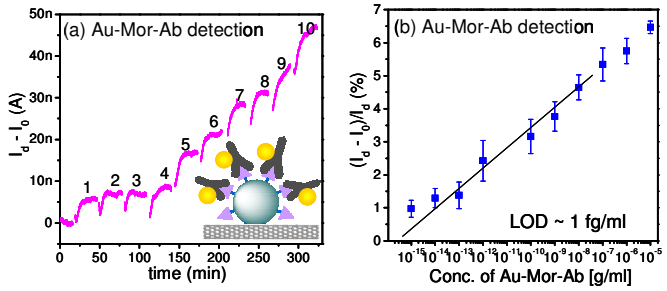

Figure 4: Indirect detection scheme where MAM-BSA is used as the receptor for Au-Mor-Ab detection. (a) Kinetic measurement plot (b) Concentration plot

4 CONCLUSIONS

We have demonstrated that by adopting the indirect detection approach using the MAM-BSA as the receptor for Mor-Ab detection, a LOD of ~130 fg/ml was achieved. This limit was further improved down to ~ 1 fg/ml by incorporating AuNPs into the sensing system. The signal amplification was a result of the increased carrier concentration owing to the electrostatic gating influence from the negatively charged AuNPs and the MAM-BSA: Mor-Ab interaction.

It may be noted that the detection of Au-Mor-Ab is only the first step toward the detection of heroin family, since ultimately the analytes of interest is free MAM molecules instead of its antibody counterpart. A combination of competitive immunoassay with indirect detection approach should therefore be employed for the ultimate MAM detection [16].

REFERENCES

[1] N. Sinha, J. Ma, J.T.W. Yeow, Journal of Nanoscience and Nanotechnology, 6, 573, 2006.

[2] M.P. Anantram, F. Leonard, Reports on Progress in Physics, 69(3), 507, 2006.

[3] Y. Cui, Q. Wei, H. Park, C.M. Lieber, Science, 293(5533), 1289, 2001.

[4] K. Bradley, J.-C.P. Gabriel, M. Briman, A. Star, G. Grüner, Phys. Rev. Lett. J1 - PRL, 91(21), 218301, 2003.

[5] R.J. Chen, S. Bangsaruntip, K.A. Drouvalakis, N. Wong Shi Kam, M. Shim, Y. Li, W. Kim, P.J. Utz, H. Dai, PNAS, 100(9), 4984, 2003.

[6] S. Gandhi, P. Sharma, N. Capalash, R.S. Verma, C.R. Suri, Analytical and Bioanalytical Chemistry, 392, 215, 2008.

[7] P.P. Dillon, B.M. Manning, S.J. Daly, A.J. Killard, R. O'Kennedy, Journal of Immunological Methods, 276(1-2), 151, 2003.

[8] J.N. Tey, I.P.M. Wijaya, Z. Wang, W.H. Goh, A. Palaniappan, S.G. Mhaisalkar, I. Rodriguez, S. Dunham, J.A. Rogers, Applied Physics Letters, 94(1), 013107, 2009.

[9] J.N. Tey, I.P.M. Wijaya, Z. Wang, W.H. Goh, A. Palaniappan, S.G. Mhaisalkar, I. Rodriguez, S. Dunham, J.A. Rogers, Appl. Phys. Lett., 94, 013107, 2009.

[10] Y. Zhou, L. Hu, G. Gruner, Appl. Phys. Lett., 88, 123109, 2006.

[11] I. Heller, A.M. Janssens, J. Mannik, E.D. Minot, S.G. Lemay, C. Dekker, Nano Lett., 8(2), 591, 2008.

[12] E.D. Minot, A.M. Janssens, I. Heller, H.A. Heering, C. Dekker, S.G. Lemay, Appl. Phys. Lett., 91(9), 093507, 2007.

[13] N.H. Thomson, Journal of Microscopy, 217(3), 193, 2005.

[14] L. Torsi, G.M. Farinola, F. Marinelli, M.C. Tanese, O.H. Omar, L. Valli, F. Babudri, F. Palmisano, P.G. Zambonin, F. Naso, Nature Materials, 7(5), 412, 2008.

[15] M.T. Castañeda, S. Alegret, A. Merkoçi, Electroanalysis, 19(7-8), 743, 2007.

[16] J.N. Tey, S. Gandhi, I.P.M. Wijaya, A.Palaniappan, J. Wei, I. Rodriguez, C.R. Suri, S.G. Mhaisalkar, Small, Accepted, 2010.