Study Of The Correlations Between Direct Electron Transfer Rate Constants And The Effectiveness Of Cancer Inhibitors Using Nanobiomimetic Sensors

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ABSTRACT

We report a new systematic approach to study Direct Electron Transfer (DET) rate constants between an electrode surface and an active biomimetic receptor site using four types of gold nanobiomimetic sensor chips. The DET results were obtained under antibody-free, label-free and analyte-free conditions in cell culture media using a cyclic voltammetry (CV) method. Sensor 1 has 20 nm nanopores, Sensor 2 has 40 nm nanopores, Sensor 3 has nano islands and Sensor 4 has a flat nano bridge with nanopores. The DET k_s results are sensor 4>sensor 3>sensor1>sensor 2. Anti-cancer compounds **1**-Methyl-2-piperidinemethanol (MPPDM), Flavopiridol hydrochloride hydrate (FLAPHH) and o-nitrophenyl acetate (o-NPA) were used for evaluation of brain cancer cells of SNB-19 by a Chronoamperometric (CA) method and a CV method. Results shown the MPPDM and o-NPA completely blocked cancer signaling with sensor 4 at 5 cancer cells/mL, while sensor 3 was unable to detect at this level. Cancer detection rate is 97 ± 6% and 90 ± 4% for a CV and a double step chronopotentiometry (DSCPO) method, respectively at 50 cell/mL using cancer spiked NIST human standard sera SRM 965A against that of the samples in the media. Our preliminary results revealed the higher DET values may have a positive correlation to the effectiveness of the anti cancer compounds.

Keywords: Nanobiomimetic sensing; Direct Electron Transfer (DET); Electron-relay; Biomimetic "ATP Lid";

INTRODUCTION

Label-free live cancer cell detection technologies have drawn attentions [1-3]. The single live cancer cell can be sensitively detected is of very important for early-stage cancer screening. Electrochemical impedance spectroscopy method used to detect single cancer cell was reported [4]. E. Chen's group reported a nanobiomimetic electrochemical device can selectively and quantitatively detect live single breast cancer cell MDA-MB-231 line with a double step chronopotentiometric (DSCPO) method under the antibody-free and label-free conditions, most importantly is the method offered fast direct detection within milliseconds and seconds range without any sample preparation and assay for protein marker conjugation. The method is solely based upon the direct bio-communication between the cancer receptors and the Biomimetic receptors of the sensor membrane [5-7]. The results of the DSCPO method were further validated by a chronoamperometrc method [7].

Overcoming protein non specific binding has been a long history of battle in the biotechnology, pharmaceutical and *in vitro* diagnostic industries [8-11]. The well accepted and commonly used terminology "**Direct Electron Transfer**" (DET) appeared in electrochemical sensor literature that refers to the electron direct transfer between redox centers of an enzyme and the surface of an electrode without using a mediator or probe [12**]**. Colloidal nano-gold particles have been extensively studied for the utility of promoting direct electron transfer (DET) between enzymes and the nano-particles [13-14].

Study of DNA charge transport may reveal important biological molecule signaling information, however, the low charge transport rate, protein conformation change and time consuming of labeling procedures are burdensome [15-16]. Biomimetic electron-relaying system, which not only mimics the active sites of the proteins, but also promotes direct bio-communication between the artificial active sites and the electrode by utilizing a nanostructured self-assembled membrane (SAM) films to enhance the sensor selectivity, sensitivity and environmental protectiveness. It was discovered that the structures of biomimetic enzyme sensor membranes played an important role in enabling selectively detecting of toxins for being able to distinguish isomers [17]; For being able to detect biological metabolic molecules to distinguish the isomers of blood glucose [11,18], and to detect live cancer cells between different types of cancers [19] without the presence of antibody and labeling. Study of the correlations between the DET rate constants and the effectiveness of the anti cancer compounds is the goal of this research.

EXPERIMENTAL

Fabrication of the Nanostructured Biomimetic PDC Conformational "ATP Lid" Self-Assembling Membranes (SAM)

Sensor 1 SAM was prepared according to the published procedures based on a cross linked conductive polymer of the mono imidazol derivative dimethyl ß-cyclodextrin (mM-ß-DMCD), polyethylene glycol diglycidyl ether (PEG) and poly(4-vinylpyridine) (PVP) [11, 18]. The mM-ß-DMCD was synthesized according to the published procedures [21]. **Sensor 2** SAM was fabricated by cross-linking (PEG), triacetyl-ß-cyclodextrin (T-CD) and β-CD/epichlorohydrin according to a published procedure [7]. **Sensor 3** was fabricated by cross-linking of PEG, T-CD, PVP and ß-CD copolymer with appropriate amount of propositions [18]. The nanostructured biomimetic PDC SAM with the flat bridged conformational "ATP Lid" was freshly prepared by adding appropriate amount of o-nitrophenyl acetate (o-NPA) embedded into the mixture solution of mM-ß-DMCD, T-CD, PEG and PVP, and incubation conditions were similar as above mentioned procures. This last sensor SAM as **Sensor 4** was used for this study.

Characterization of the Membrane

The morphology of the AU/SAM was characterized using a Atomic Force Microscope (AFM) (model Multimode 8 ScanAsyst, Bruker, PA). Data Collected in PeakForce Tapping Mode. Probes used were ScanAsyst-air probes (Bruker, PA). The silicon tips on silicon nitride cantilevers have 2-5 nm radius. The nominal spring constant 0.4N/m was used. Figure 1 illustrates the 3D vertical conformational PDC bridge structure with "breathing nanopore" of the AFM images of the Biomimetic "ATP Lid". Figure 2 illustrates the 3D flat conformational PDC bridge structure with "breathing nanopore" of the AFM images of the Biomimetic "ATP Lid"

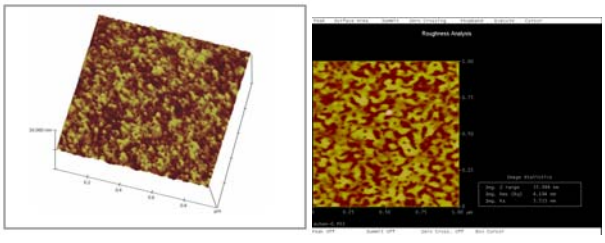


Fig 1 AFM images of Sensor 1 with an average 20 nm nanopore SAM (L) and Sensor 2 with an average 40 nm nanopore SAM (R)

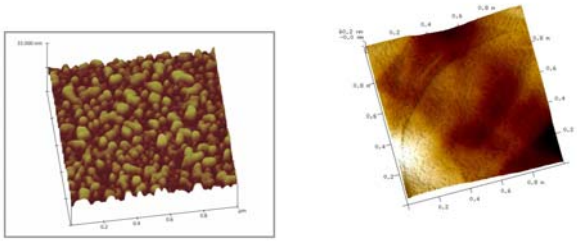


Fig 2. AFM images for Sensor 3 with islands structure (L) and Sensor 4 with a flat bridge structure (R).

Human Brain Cancer Line SNB-19

The glioblastoma brain cancer cells samples are human neuro blastoma line SNB-19 as shown in Fig 2 (right). The cell cultures were held in a base growing medium of DMEM (Dulbecco/Vogt Modified Eagle's minimal essential Medium – a common growth culture medium used for human cell incubation) (Invitrogen, CA infused with a 10% concentration of FBS (fetal bovine serum), 10 mM HEPES, 100 units/mL penicillin/Streptomycin and 2 mM L-glutamine. It was kept in a normal atmosphere at a temperature of 37.0 ºC with 10% CO_2 and humidified air. The cancer cells in the DMEM media were incubated for 24 hrs. Before test the cancer cells, dilution procedures were conducted.

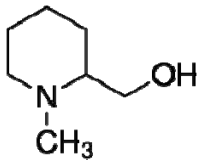
DET

DET rate constant (K_s) can be obtained in a sensor system by changing the scan rate of the cyclic voltammetry in a media without the presence of an analyte [20]. The equations used for the K_s are from Laviron's equations [21]. The four sensors were tested in DMEM cell culture solutions in room temperature with changes of scan rates.

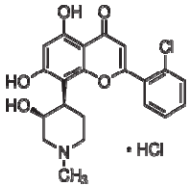
Effectiveness of Cancer Inhibitors

Effectiveness of anti-cancer compounds was assessed in vitro with known amount of cancer cells spiked in the culture solutions with know concentrations of anti cancer compounds, that used against control without cancer cell using an amperometric method. The ranges of cancer cells are over 1 to 100 cell/mL using an electrochemical work station (Epsilon, BASi, IN). **"Point Accuracy"** was checked by using a NIST certified standard human sera SRM 965A with known level of glucose and spiked with known amount of live cancer cells with or without anti cancer compounds. Samples run triplicates. The point accuracy defined as the ratio of the average signal intensity of the spiked cancer cells in the NIST SRM 965A sera against that of the average signal in media at the given cancer concentration. The anti cancer compounds are: 1-methyl-2-piperidinemethanol (MPPDM), it is a dual vascular endothelial growth factor

receptor-2 and fibroblast growth factor receptor 1 inhibitors as an anti cancer compound and flavopiridol hydrochloride hydrate (FLAPHH) is a potent cyclin-dependent kinase (CDK) inhibitor. A potential candidate of inhibitor is o-nitro phenyl acetate.



1-Methyl-2-piperidinemethanol (MPPDM)



Flavopiridol hydrochloride hydrate (FLAPHH)

RESULTS AND DISCUSSIONS

DET

According to the commonly used E. Laviron's method (21), the rate constants were calculated based on the irreversible and reversible situations exist differently, hence equations were used according to each special CV profile. Fig 3 illustrates the quasi-reversible CV profiles, and the linear relationship of the cathodic or anodic peak currents with scan rates suggests the heterogeneous surface controlled electron transfer process. The K_s obtained is 92/s for Sensor 1. Sensor 2 was failed to observe the existence of DET peak (figure not shown). Fig 4 illustrates the CV profiles for Sensor 3. Voltage vs log scan rate is linear suggests the DET transfer occurred in the interface as shown in Fig 4(L). The K_s value calculated is 185/s. Fig 5 Illustrates Sensor 4's CV profiles for DET and plot of current vs. scan rate shown as the insert. The K_s value calculated is 274.5/s for Sensor 4 with the flat bridged nanostructure membrane. The rank of the K_s values are Sensor 4> Sensor 3> Sensor 1 (Sensor 2 is unavailable).

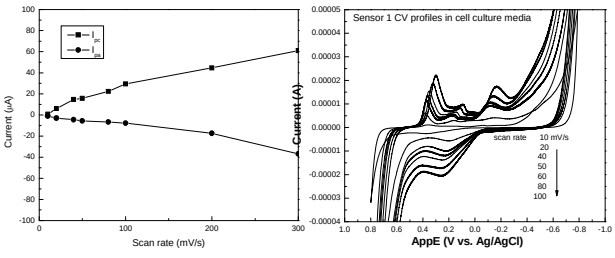


Fig 3 Illustrates Sensor 1's CV profiles for DET in culture media (R) and plot of current vs. scan rate as shown (L).

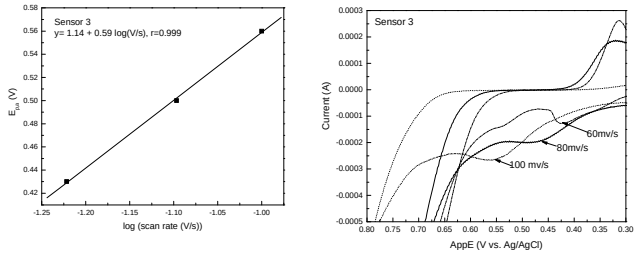


Fig 4 Illustrates a plot of voltage vs. log scan rate (left) and CV profiles at right.

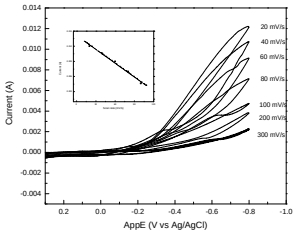


Fig 5 Illustrates Sensor 4's CV profiles for DET and plot of current vs. scan rate shown as the insert.

Effectiveness of Cancer Inhibitors

The K_s values obtained from the DET study have paved the road for assessing the effectiveness of the anti-cancer compounds, therefore Fig 6 illustrates the profiles of Sensor 3 detects brain cancer cells with or without anti cancer compounds under the conditions of no incubation. Fig 7 illustrates the effectiveness of the anticancer compounds to diminish the 2000 cancer cell/mL signaling compared with the control. The 1% precision was for all cases except for the cancer cell only, which is 5%. Fig 8 illustrates the CV profiles with anti cancer compounds at 5 brain cancer /mL concentration using Sensor 4 compared with the control. The imprecision at 5 cell/mL is 8%. Using NIST's standard sera with spiked 50 brain cancer cells received a recovery of 60% which is two-fold higher than the reported data [22].

It was noticed from Fig 8 that the 5 and 1 brain cancer cell/mL signaling was completely diminished by only using a 150s electric pulse with the strength of 100 μA stimulated, respectively by Sensor 4, that indicates the Sensor 4 may have a potential to be an therapeutic device for cancers. The 20 μM MDDPH and o-NPA had completely diminished the cancer cells signaling as shown in Fig 8 and about 95% diminished at 1 cell/mL in Fig 9 . The FLAPHH has a small peak at -0.425 mV and also has 90% blocked the signaling at 1 cell/mL. Results indicate Sensor 4 provided a viable tool to fast assess the effectiveness of anti cancer compounds and therapeutic devices in vitro.

"Point Accuracy". The point accuracy defined as the ratio of the average signal intensity of the spiked

cancer cells in triplicates in the NIST SRM 965A sera against that of the average signal in media. The detection rate (Point Accuracy) using the CV method at 50 cell/mL was 97 ± 6%. The imprecision were 0.5% and 5% for media spiked and sera spiked samples, respectively. It was 3-fold higher than the literature [22]. By using the DSCPO method at the same concentration, the point accuracy is 90 ± 4% error; the imprecision values are 2% and 3% for spiked NIST sera and media samples, respectively. There was a good agreement of the point accuracy between the two methods.

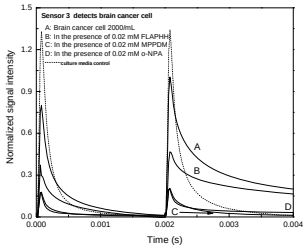


Fig 6 Illustrates the profiles of a CA method with or without anti cancer compounds with Sensor 3.

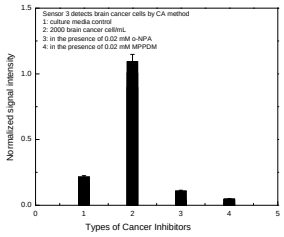


Fig 7 Illustrates the effectiveness of the anti cancer compounds using Sensor 3.

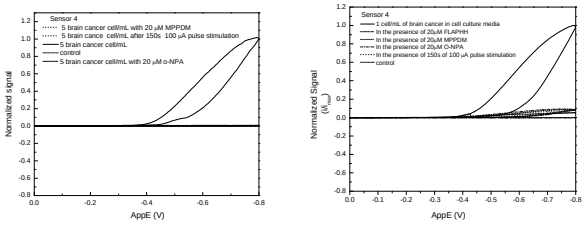


Fig 8 Illustrate the effectiveness of cancer inhibitors at 5 (L) and 1 brain cancer (cell/mL) using Sensor 4.

CONCLUSIONS

This work provides a useful means to assess the effectiveness of anticancer compounds with DET. Our results revealed the higher DET values may have a positive correlation to the effectiveness of the anti cancer compounds. Our preliminary results provide a relatively high cancer detection rate based upon the simplicity, sensitivity and selectivity of the nanobiomimetic sensing platform technology. Further study of the clinical merits using *in situ* heterogeneous specimens is needed for seeking the real world applications.

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