Nanostructured Biomimetic Pyruvate Dehydrogenase Complex (PDC) Sensors Selectively Detect Single Brain Cancer Cell And Have The Ability To Mimic The "ATP Lid"

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ABSTRACT

Diseases such as cancer, diabetes mellitus, and brain injury can be caused by lack of conformational change in the pyruvate dehydrogenase complex (PDC). The known "ATP Lid" for the function of PDC is comprised by the complex of L2 forming a flat bridge with PDK3 nanopore structured subunit. We report the new nanobiomimetic PDC sensors having the ability to detect single brain cancer cell of SNB-19, while rejecting breast cancer cells of MDA-MB-231 from 1 to 100 cell/mL without antibody and labeling. Three gold sensors were developed and studied by cyclic voltammetry (CV) and chronoamperometric (CA) methods. Studies of selectivity were based on 2 types of cancer cells detections using Sensor 1 (with a vertical bridge structure with nanopore breathing core) compared with Sensor 2 (with a flat bridge structure and nanopore). Sensor 3 with a nanopore only structure also considered. Results shown live glioblastoma cancer cells have strong structure preference of a flat bridge structure over a vertical one with a Limit of Detection $1.2x10^{-18}$g.

Keywords: Nanobiomimetic sensing; Biomimetic pyruvate dehydrogenase kinase (PDK)3 and lipoyl binding 2 pocket; Hydrophobic interaction; Selectivity.

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 selectively and quantitatively detected live single breast cancer cell MDA-MB-231 line with a double step chronopotentiometric (DSCPO) method under the antibody-free and label-free conditions. The method offered fast direct detection of a single cancel cell within milliseconds and seconds range without any sample preparation 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]. Furthermore, the heat released from cancer cells is an order of magnitude higher than normal breast cell using a multiple variable Contour Map method reported by Chen's group [5].

Overcoming protein non specific binding has been a long history of battle in the biotechnology, pharmaceutical and *in vitro* diagnostic industries [8-11]. Excellent reviews and articles of enhancing the selectivity of various artificial enzyme models for drug designs and for *in vitro* diagnostic devices were reported elsewhere [8-10]. Our group's works in developing nanostructure biomimetic electrochemical biosensors with high sensitivity and selectivity having the capability to distinguish isomers of niotrophenyl acetate, to distinguish isomers of D-glucose under label-free, reagent-less and antibody-free conditions were reported [11-13]. However, confirmation of the nanostructured biomimetic sensors selectively detection of different types of cancer cell lines in single cell concentration is a great challenge. Turning our focuses to learn from nature may find an answer. The target protein is Pyruvate Dehydrogenase Complex (PDC). PDC exists mainly in mitochondrial cells in the brain and kidney and reproductive organs, and PDC is the key mitochondrial metabolic enzymes and it is responsible for the production of cellular energy in the form of ATP. Three-dimensional structure analysis of PDC revealed its highly symmetric structure exists with a hollow truncated cube with an edge of 125A, forming the core of the multienzyme complex [14]. Crystal structures of PDC revealed from D. T. Chuang's group that the C-terminal tail from one subunit of PDK3 dimer constitutes an integral part of the lipoyl-binding pocket in the N-terminal domain of the opposing subunit. The two swapped C-terminal tails promote conformational changes in active-site clefts of both PDK3 subunits, resulting in largely disordered ATP lids in the ADP-bound form. The data suggest that L2 binding stimulates PDK3 activity by disrupting the ATP lid, which otherwise traps ADP, to remove product inhibition exerted by this nucleotide [15]. The hydrophobic interactions occur mainly in the lipoyl binding pocket in PDK3. These structural components

formed a deep cylindrical pocket with hydrophobic residues. There is a dynamic equilibrium between completely disordered vertical ATP lid and a relatively ordered flat ATP lid for the purpose of easily release ADP and for trap ADP [15]. Z. H. Zhou's paper also revealed the flexible bridge exists within the truncated E2 core [16].

It is a well known phenomenon that over expression of function of PDK isomers in various tissues leads to various diseases, such as cancer, diabetes mellitus, heart ischemia and brain injury [17-20]. Unfortunately, lack of real time in situ monitoring the conformational change of the PDC with anticancer compounds, or with live cancer cells monitoring has hampered the pharmaceutical therapy developments. Our approach is to use a "Let the chicken go free" strategy, i.e., under antibody-free and labeling-free conditions, let the cancer cell freely communicate with the sensor receptors, therefore the interference will be drastically reduced and the non specific bounding will be reduced based on the direct bio-communication. This is the goal of this study.

EXPERIMENTAL

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

The nanostructured biomimetic PDC SAM with the vertical bridged conformational "ATP Lid" was freshly prepared. Polyethylene glycol diglycidyl ether (PEG), triacetyl-ß-cyclodextrin (T-CD), poly(4-vinylpyridine) (PVP) were purchased from Sigma. PVP was purified before use. The mono imidazol derivative dimethyl ß-cyclodextrin (mM-ß-DMCD) was generally synthesized according to the published procedures [21]. The appropriate amount of solutions of individual polymer and reagents were prepared [7]. The mixture solution was made up by mM-ß-DMCD, T-CD, PEG and PVP. The 16 channels gold electrode chip was purchased (Fisher Scientific). The mixture solution was injected onto the surface of the electrode and was incubated for 48 hrs at 37ºC [7]. After that, the further clean and incubating procedures were followed by literature 7. This Au/SAM used as for sensor 1.

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) into the above described mixture solution for the vertical bridged PDC SAM. All other procedures were followed as cited in literature 7. This Au/SAM used as for Sensor 2. Sensor 3 was fabricated based on a cross linked conductive polymer of mM-ß-DMCD, PEG and PVP [11]. It has a uniform nanopore arrayed 3D structure, but without a bridge feature.

Characterization of the Membrane

The morphology of the AU/SAM was characterized using an 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"

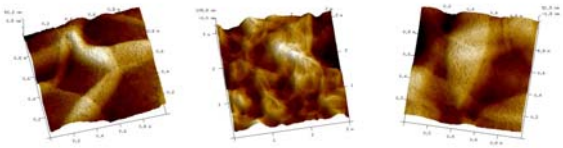

Fig 1. 3D vertical conformational PDC bridge structure of the AFM images of the Biomimetic "ATP Lid".

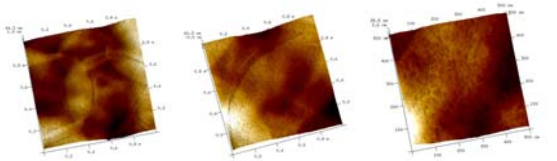

Fig 2. 3D horizontal conformational PDC bridge structure of the AFM images of the Biomimetic "ATP Lid".

Human Breast Cancer Cell Line MDA-MB-231 and the Glioblastoma Brain Cancer Line SNB-19

Breast cancer cell samples are human adenocarcinoma cells line MDA-MB-231 as shown in Figure 3 (Left) taken from breast cancer tissue. The glioblastoma brain cancer cells samples are human neuro blastoma line SNB-19 as shown in Fig 3 (right). The cell cultures are 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.

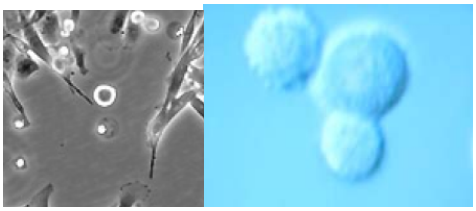

Fig 3 Illustrates the breast cancer cell image (left). The brain cancer cell image (right).

Selectivity

The selectivity study was conducted at room temperature by a Cyclic Voltammetric method (CV) and a Chronoamperometric method (CA) to detect the breast cancer cells and the brain cancer cells. The scan rate was constant at 20 mV/s for the CV method.

Quantitation

The results obtained from the selectivity study were used to conduct the quantitation study. Sensor 2 with a flat bridge "ATP Lid" and a "breathing pore" conformational structure was chosen for quantitation of live brain cancer cell line by using a CV method. Sensor 3 without an "ATP Lid" but with a nanopore structure was chosen for quantitation of live breast cancer cell line in cell culture media at room temperature using a chronoamperometric method. The ranges of cancer cells are over 1 to 100 cell/mL using an electrochemical work station (Epsilon, BASi, IN).

RESULTS AND DISCUSSIONS

Selectivity of the "ATP Lid" conformations

Figure 4 illustrates the live brain cancer cell favored the flat "ATP Lid" bridge structure (Sensor 2) than that of the vertical bridge structure (Sensor 1). We were able to repeat the observations for this phenomenon. The signal intensity for the flat bridge structure is magnitudes higher than the vertical bridge structure indicated monitoring real time live cancer cell bonding conformational change is important, especially beneficial for anticancer therapeutic compound developments. Fig 4 also demonstrates its discrimination between brain cancer and breast cancer at 1 cell/mL concentration.

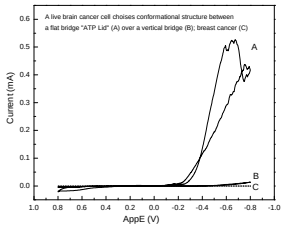

Fig 4 Illustrates the brain cancer cell selectively bonds Sensor 2 with a flat "ATP Lid" bridge structure (A). The cancer cell bonds to Sensor 1 with a vertical "ATP Lid" structure (B); Breast cancer cell (1 cell/mL) (C).

The same observations are also obtained in Sensor 1, that has the discrimination of breast cancer cell at 1 cell/mL concentration (data not shown). It is shown in Fig 5, that Sensor 3 only selectively detects breast cancer cell over brain cancer cell, that indicates a flat "ATP Lid" structure is more important to active the brain cancer receptor than to activate a breast cancer cell due to o-NPA enhanced the Biomimetic PDC's hydrophobic π- π stacked pocket [15].

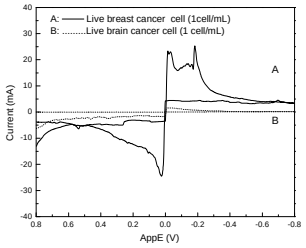

Fig 5 Illustrates Sensor 3 selectively detecting live single breast cancer cell (1 cell/mL) over live brain cancer cell (1 cell/mL) after 24 hrs incubation.

Selectivity Using An Amperometric Method

Selectivity was further confirmed by an amperometric method using Sensor 3 as shown in Figure 6. It demonstrates Sensor 3 selectively detect breast cancer cell over brain cancer cell at 1cell/mL concentration.

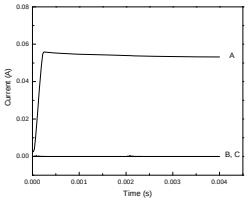

Fig 6 Illustrates the ameprometric profiles using Sensor 3. (A) breast cancer cell with incubation; (B) brain cancer (BC) cell with incubation; (C) BC without incubation.

Quantitation of Cancer Cells

The results obtained from the selectivity study enabled us to conduct the quantitation study. Sensor 2 with a flat bridge "ATP Lid" and a "breathing pore" conformational structure was chosen for quantitation of live brain cancer cell line by using a CV method. Sensor 3 without an "ATP Lid" but with a nanopore structure was chosen for quantitation of live breast cancer cell line as shown in Figure 7 and 8, respectively. Sensor 3 in Fig 8 generates a linear regression equation of y=0.4 + 0.08x, r

=0.995, Sy/x= 0.46 over the range 1-100 cell/mL with the sensitivity of 80 μA/cell.mL^{-1}. Sensor 2 in Fig 7 generates a linear regression equation of y=0.4 + 0.07x, r=0.9992, Sy/x= 0.19 with the sensitivity of 70 μA/cell.mL^{-1}.

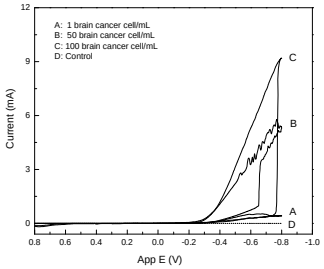

Fig 7 Illustrates the CVs of Sensor 2 for brain cancer cell at 1 (A), 50 (B), 100 cell/mL (C) and control (D).

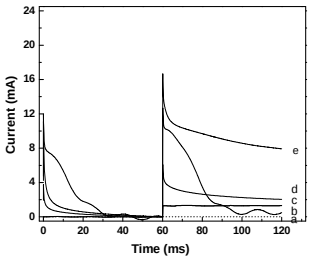

Fig 8 illustrates CAs of Sensor 3 with breast cancer cells after incubation (a) control; (b) 1; (c) 5; (d) 50; (e) 100 cell/mL.

CONCLUSIONS

This work has demonstrates live glioblastoma cancer cells have strong structure preference between "ATP Lid" with a flat bridge conformational structure over a vertical structure for activation of the L2 domain stimulated PDK3 activity. With negligible interference advantages offered during the protein dynamic activation in the center active pocket, that enabled a quantitation of early cancer detection down to a single cell, i,e. this method offered a potential means for detecting circulated cancer cell at 2 cell/L, in other word, it will detect virus and cancers in 1.2x10^{-18}g (a HIV virus is 8x10^{-16}g) [22]. This method offered orders of magnitudes sensitive of Limit of Detection (LOD) value than reported Whispering-gallery-mode label-free optical biosensor in literature [22].

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