A Nanopore Biomimetic Device Quantitatively Detects Early Stage Cancer Cells; a Contour Map of Multiple Variable Correlation Method Assesses the Heat Released by Cancer Cells

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ABSTRACT

We report a portable early stage low abundance cancer cells detection device that has the capability to detect the breast cancer MDA-MB-231 line in a single cell concentration quantitatively using the unique land marker ratio of "action potential" vs. "resting potential" (RAPRP) values that distinguish from the normal breast cells under antibody-free and labeling-free conditions. The device is based on a nanopore biomimetic cross linked polymer membrane fabricated on gold chips. This real time potential was recorded using a double step chronopotentiometry (DSCPO) under a fixed current. The sensitivity of the device to detect cancer cells is 530 mV/cell (mL^{-1}) over 1-50 cell/mL range after 24 hrs incubation. Factors of cancer cell concentration and current affect on the DSCPO profiles are reported. The heat released by the cancer cells is an order of magnitude higher in calories compared with a normal breast cell assessed by a Contour Map of Multiple Variable Correlation method (CMMVC).

***Key Words*:** Nanopore Biomimetic membrane; ratio of action potential and resting potential; RARP; Contour Map Multiple Variable Correlation Method; CMMVC; quantitatively detects low abundance cancer cells; Antibody-free and labeling-free testing; cell heat release.

INTRODUCTION

It is a well recognized phenomenon that cancer cells have abnormal cell membrane potential [1-3]. The conventional biopotential method used for diagnosing cancer is lacking in sensitivity and selectivity [3]. Biologists measure cell membrane action and resting potentials with burdensome instrumentation and time consuming procedures. A recent report shows breast cancer cell division caused a membrane potential increase [4] due to variations in ion channel expression. However, the method requires a time consuming large computer algorithm for modeling, and still lacks selectivity and sensitivity. A recent paper reported that the measured neural cell membrane spiking potential has a signal to noise ratio of 2 [5]. Because the normal cell membrane action potential is 58 mV, and -70 mV is for the resting potential [6], the small signals are very easily buried in the background noises [7] that can cause problems to pediatric neurologist and intensive care unit doctors who need strong signals to monitor and diagnose the neonatal neurological diseases [7]. There is very few, if any, to build a device that can induce receptors of cancer cells spontaneous and direct interact with the artificial receptor of the membrane of the device without using antibody or labeling. The amplified signals are several orders of magnitude higher in signal to noise ratio than the conventional methods, will provide means to enhance the sensitivity and selectivity of the detection. The goal of this project is to develop such a device by fabricating a nanopore structured biomimetic membrane on a gold chip with an imidazolium receptor in the polymer network to induce the direct biocommunication to cancer surface receptors without using antibody, and without labeling in order to overcome the current technology drawbacks.

EXPERIMENTAL

Fabrication of the Nanostructure Self-Assembling Membrane (SAM) Gold Sensor Chip

Reagent grade poly (4-vinylpyridine) (PVP), polyethylene glycol diglycidyl ether (PEG), were purchased from Aldrich-Sigma. The PVP was recrystallized in methanol. The mono imidazol derivative dimethyl ß-cyclodextrin (mM-ß-DMCD) was generally synthesized according to the published procedures **[8].** The gold chips were purchased (Fisher Scientific) and the mixture solutions with proper compositions and procedures were followed by published literature in **[9].**

Characterization of the Membrane of AU/SAM

The morphology of the AU/SAM was characterized using a Dimension 3100 Atomic Force Microscope (AFM) (Bruker Nano, CA). The surface structure, shown in Figure 1, was scanned by TappingMode AFM using a silicon cantilever and a tip with a 5-10 nm radius and resonance frequency of 300kHz [10]. The roughness of the SAM was

0.82 nm RMS. Figure 2 is an art illustration of the model used to construct the Au-nanopored sensor by SAM method.

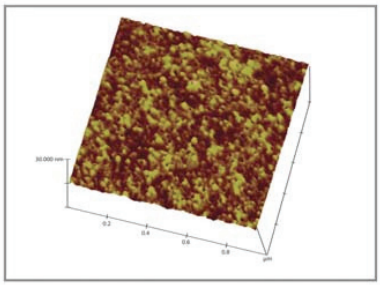

Figure 1. An AFM 3D image of the glucose Sensor with nanopore structure and an internal artificial receptor.

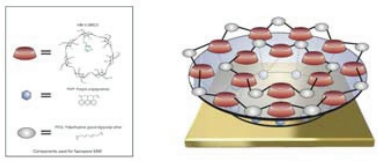

Figure 2. An art illustration of the work for the model used to construct the SAM/Au-nanopored sensor.

Human Cancer Cell Line MDA-MB-231

Breast cancer cell samples are human adenocarcinoma cells as shown in Figure 3 taken from breast cancer tissue. 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) infused with a 10% concentration of FBS (fetal bovine serum). It contains 4.5 g/L glucose, sodium bicarbonate 1.5g/L and 4 mM L-glutamine. It was kept in a normal atmosphere at a temperature of 37.0 ºC with 10% CO_2. The culture requires medium renewal 2-3 times per week. The cancer cells in the DMEM media were 100 k cell/mL with or without incubation as shown in low cell density in Figure 3. The incubation cell samples were incubated for 24 hrs in CO_2. Before test the cancer cells, dilution procedures were conducted by diluting the high concentrated cell solution with the culture media. Hence beast cancer cell concentrations of 1, 5, 50, 100 and 200 cell/mL were prepared.

Selectivity

The selectivity of the sensor towards detecting the breast cancer cells compared with that of normal living breast cells were conducted at room temperature by the DSCPO method. The normal breast specimen was tested by the DSCPO method in a non-invasive manner, that the wetted sensor was directly attached on the skin of the breast of the subject, whom was consent and was approved with the IRB.

Double Step Chronopotentiometry (DSCPO)

The DSCPO method was used for evaluation of the sensor performance for cancer detection under fixed current conditions. Changes of current effects on the "action potential" and "resting potential" were conducted in the range from pA to mA in vitro culture medium at room temperature. All experiments were finished within 1 hr. Changes of cell concentrations effect on the potentials were conducted in the ranges from 1, 5, 100, to 200 cell/mL using an electrochemical work station (Epsilon, BASi, IN). The 16 channel AU/SAM electrode chip configuration was mentioned in *Section of Fabrication of the Nanostructure Self-Assembling Membrane (SAM) Gold Sensor Chip.* The center circular electrode is the working electrode, and the adjacent gold electrodes are the auxiliary and the reference electrode, respectively. The discharge potential was defined as "action potential", and the charge potential defined as "resting potential. The duration time is 2s for action or resting potential for the model cancer sensor study. The absolute value of action potential divided by the resting potential was defined as the ratio of action potential vs. resting potential. The ratio was used for assess of cell heat release by a Contour Map Multiple Variable Correlation method (CMMVC).

Assessing Cell Heat Release

The CMMVC method was used for assess of cancer cell heat release. Two variables chosen for assessing the heat released by cancer cells (as Z axis) were 1. Ratio of "Action potential" vs. "Resting potential" (as Y axis) and 2. Cell concentration as X axis was used for cell concentration factor study. Similarly, it was only a change in X axis to current, while other factors are remain the same, was conducted for the current factor study. The results of absolute difference between action and resting potential at a given cell concentration under a known current, were used to multiply the current and then multiply the time duration of the potential fired by the equation of $J = I.\Delta V.t$, I is current in ampere, ΔV is voltage difference in volt and t is time in second. J is Joule. Joule divided by a 4.184 conversion factor gives the calorie released.

RESULTS AND DISCUSSIONS

Characterization of the SAM

The reported 3D view of nanopore structured multiple-function CD-SAM is shown in Figure 1. The AFM

image clearly reveals the smoothness of the SAM. The nanopores were well distributed and vertically oriented on the gold surface with a pore size from 10 to 20 nm, and the roughness of the SAM was 0.82 nm RMS. Figure 2 shows an art illustration of the work for the model used to construct the Au-nanopored His 516 receptor-cyclodextrin (CD) SAM electrode. The moiety of the receptor-CD was cross linked with polyethylene glycol diglycidyl ether (PEG) and poly(4-vinylpyridine) (PVP) and self-assembled a nanopore structured SAM through hydrogen bonding [10].

DSCPO Profiles

Effect of Current Change with or Without Cell Incubation

Figure 4 and 5 illustrate current change effect on the DSCPO profiles under 5 cell/mL concentration with or without 1 day incubation, respectively, against the controls that did not have cancer cell. Both figures were without inhibitors. It is obvious that with 1 day incubation, the DSCPO's action and resting potential profiles moved up to all positive potential fields, especially for resting potential, indicating the cancer cells are not in a normal "resting potential" stage, i.e, -70 mV, have critically impacted the ratio of action/resting potential (RAPRP), hence the results of RAPRP are larger than that of without incubation. The action potential signals were increased as current increased drastically than that of without incubation. For without incubation, there were superimposed curves for resting potentials regardless the current changes. Current changes had smaller impact on the potentials for without incubation than that of with 24 hrs incubation.

Effect of Current Change on a Normal Breast Cell

A current change effect on a living normal breast cell was illustrated in Figure 6. The amplitude of curves at the action and resting potential fields are symmetric alone the zero line, indicating the RAPRP values are close to 0.75-0.9 range, which is at a normal electrophysiological situation [6]. This sensor demonstrated its capability to selectively induce a bio communication more favorably to cancer cell rather than to a normal cell at very sensitive concentration level, because cancer cells with high negative charge density tend to direct hydrogen bonding to the positive imidazolium receptor in the sensor membrane.

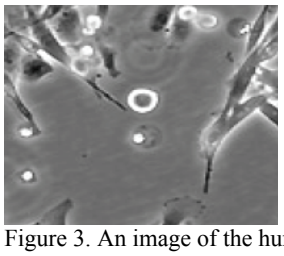

Figure 3. An image of the human breast cancer cells of MDA-MB-231 in a base growing medium of DMEM.

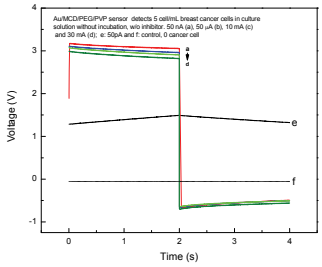

Figure 4. Illustrates the effect of current on DSCPO profiles without incubation and without inhibitor under 5 cancer cells/mL concentration with current change from a to d: 50 nA, 50 μA, 10 mA, 30 mA; e: 50 pA, f: control without cell;

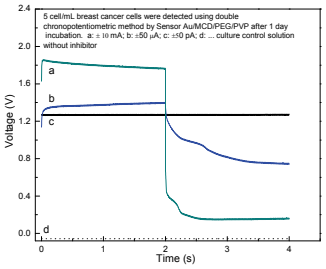

Figure 5. Illustrates the effect of current change on DSCPO profiles with 1 day incubation and without inhibitor under 5 cancer cells/mL concentration with current change from 10 mA (a), 50 μA (b), 50 pA (c) and without cancer cell (d).

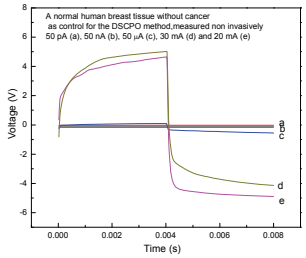

Figure 6. Current change effects on the DSCPO profiles of a normal living breast cell in a non invasive manner from a to e: 50 pA (a), 50 nA (b), 50 μA (c), 30 mA (d) and 20 mA (e), respectively.

Effect of Cancer Cell Concentration Change

The changes of cancer cell concentrations effects on DSCPO profiles were studied over the range of 1 to 200 cancer cells/mL in culture solution after 24 hrs incubation shown in Figure 7. The data fitted to an exponential model over 1 to 50 cell/mL has a 0.53V/cell. mL^{-1} increase rate for the action potential.

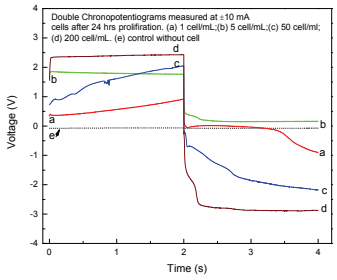

Figure 7. Illustrates the effect of cell concentration on the DSCPO profiles over 1 to 200 cell/mL range (a to d) at ±10 mA after 24 hrs incubation against a control as the dotted line (e).

Breast Cancer Cell Heat Release

The blue color CMMVC map in Figure 8 illustrates the normal breast cell heat release to the body, which is negligible. The results shown in red hot color in Figure 9 (L) and (R) are for the CMMVC visual map results for with or without incubation under 5 cancer cell/mL and ±10 mA current conditions. The difference was positively correlated with the high abnormal RAPRP as discharge current rose to mA level for cancer cells. The order of magnitude higher in the RAPRP ratio associated with more heat release is the land marker behavior of the breast cancer cells under higher current is demonstrated. In contrast, the normal breast has the RAPRP ratio close to the normal ratio range of 0.75-0.9 with no extra heat was released to the body.

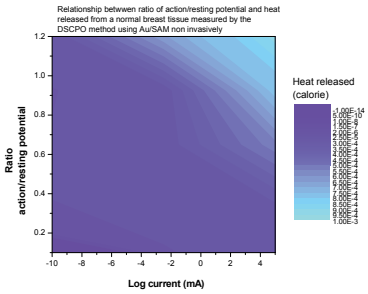

Figure 8. Illustrates the normal breast cell heat release map.

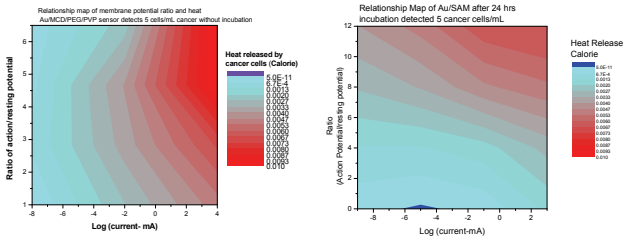

Figure 9. (L) illustrates the 5 cancer cells heat released without incubation; (R) with 24 hrs incubation.

CONCLUSION

A portable low abundance cancer cell detection device has demonstrated the capability to detect the MDA-MB-231 line of breast cancer in a single cell concentration quantitatively using the unique finger print RAPRP values that distinguish from the normal breast cells under antibody-free and labeling-free conditions. The exponential increase rate of the cell spiking potential between the cancer cell and the receptor of the membrane is 530 mV/cell. (mL^{-1}) over the range of 1-50 cell/mL. The CMMVC map method used to assess the heat released by the cancer cells opens a door for further study of various diseases with a fast, selective and convenient method.

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