Nanostructured Biomimetic Memristor Sensors Used For Therapeutic Monitoring Of The Brain Cancer

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ABSTRACT

Current technology is not available to provide a portable, simple and accurate real-time monitoring and treatment of cancers in an early stage at a clinical setting. Challenges for developing such a tool, include the need to develop an advanced sensing technology are paramount. Here we report a memristor sensor device (sensor #1) for timely monitoring and treatment of brain cancers at 5 cell/mL concentration. The purpose of this study is to determine the device's capability to monitor the heat release at a 5 cell/mL concentration and compare these results with our prior work in triple-negative (TNA) breast cancer detection results. The output of our device is a cancer cell heat release map in a 3D format which compares before and after treatment from three short pulses applied to the brain cancer cells in vitro based on a nanostructured memristor sensing technology with cross linked polymer membrane fabricated on gold chips under a reagent-free condition for monitoring of the therapeutic application as sensor #1 compared with a nanopore sensor #2 which has a selectivity for TNA cancer only. Our results shown the heat reduced by 60, 80 and 100% for sensor #1 compared with -40, 53 and 100% for sensor #2 after first, second and third pulse treatment, respectively. A preliminary non invasive, *in vivo* real time monitoring from a normal breast of a subject was conducted and the 3D heat release mapping data was presented.

Key Words: Nanobiomimetic Memristor Sensing; Electric Pulse Treatment; 3D Heat Release Mapping; Ratio of Action potential vs. Resting potential;

INTRODUCTION

Various image technologies are used for non invasive monitoring brain cancer. However, current technology is not available to provide an easy, portable, simple and accurate real-time monitoring and treatment of cancers at an early stage of only a few cancer cells at a clinical setting. Improving the biosensor performance is challenged for unavoidable biofouling and nonspecific protein bounding caused interference by utilizing nature enzyme or coenzyme [1-4].

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) film, that offers attractive pathway to enhance the 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 and different type of cancers [1, 5-8]. Memristors and Memcapacitors are devices made of nanolayers that have the capability to mimic neuronal synapse with a characteristic of hysteresis loop in the i-V curve [9-13]. Our group has developed a new type of memristor/mamcapacitor sensor used for direct detection of acetylecholine in fM level without using nature acetylecholinesterase (ACHE) under a labeling-free and reagent-less condition [14]. The detailed membrane fabrications procedures were disclosed in literature [14]. Our memristor sensor has the characteristic of hysteresis loop in the i-V curve shown in the literature [9-11, 14]. The purpose of this research is to explore the therapeutic utility of the memristor sensor device to monitor the therapeutic progress for brain cancer after stimulating electric pulse treatments. Anti cancer drugs and therapeutic treatment have been facing challenges to penetrate the blood–brain barrier (BBB). Even though our group developed a unique sensor for detection triple-negative (TNA) breast cancer at low concentration and was able to monitor cancer progress with a 3D heat release mapping method [15], however utilizing this technology for a therapeutic propose for brain cancer is still paramount.

EXPERIMENTAL

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

The memrisor device with the flat bridged conformation/nanopore was freshly prepared and fabricated on gold chips. 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 imidazolyl derivative dimethyl ß-cyclodextrin (mM-ß-DMCD) was generally synthesized according to the published procedures [16]**.** The appropriate amount of solutions of individual polymer and reagents were prepared [8]. The mixture solution was made up by mM-ß-DMCD, T-CD, PEG and PVP in an appropriate compositions and wormed up at 37°C for 2 hrs, then adding appropriate amount of o-nitrophenyl acetate (o-NPA) into the above described mixture solution used for fabricating onto the gold chips [8]. The 16 channels gold electrode chips were purchased (Fisher Scientific). This memristor used as Sensor 1. Sensor 2 is an ordinary nanopore structured Biomimetic sensor used for detection of TNA breast cancer without the memristor or memcapacitor's i-V curves, and the fabrication method was reported everywhere [15].

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. Fig 1(L) illustrates the 3D flat conformational bridge structure with "breathing nanopore" of the AFM images of the Biomimetic Memristor 1. Fig 1(R) illustrates the 3D average 20 nm diameter nanopores of the AFM images as sensor 2.

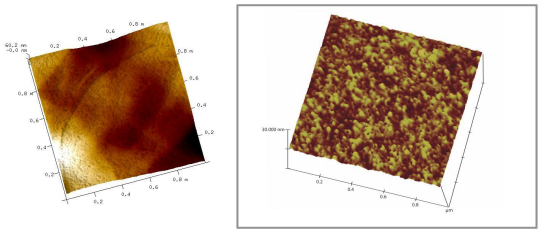

Fig 1(L) shows the 3D horizontal conformational in bridge/nanopore structure of the AFM images of the Biomimetic memristor 1. Fig 1 (R) shows the AFM image of the average 20 nm diameter nanopore sensor 2.

Characterization of the Memristor

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

The glioblastoma brain cancer cells samples are human neuroblastoma line SNB-19 and the human TNA cancer cell Line MDA-MB-231 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.

Evaluation of the Electric Pulse Therapeutic Effectiveness for Cancers

Cyclic Voltammetry (CV) Method. The electric pulses were applied onto the live cancer cells in the cell culture solution at room temperature by -10, -100, and -100 µA for each of the three pulses lasting for 50s for each using the Chronopotentiometry (CPO) method under a condition of fixed potential. After each pulse, and a CV measurement was immediately conducted at 20 mV/s against that of the control. A subject without breast cancer was used as for a baseline comparison followed the same procedure, except a mammogram was taken before and after the experiment, and follow up for 1 year recorded the mammogram changes. The experiment was approved by the board of committee.

Contour Map Multiple Variable Correlation Method (CMMVC). Our group developed the 3D CMMVC method for assessing the cancer heat release based on double step chronopotentiometry (DSCPO) method [15]. However, that was not a heat mapping method associating with time. This report has modified that method and we chose two variables for assessing the heat released (calorie as for Z axis) and 1. Gravimetric energy density was used for Y axis and 2. time was used for X axis.

RESULTS AND DISCUSSIONS

Characterization of the Memristor

The characteristics of the hysteresis loop in the i-V curve with a switch point were observed in Fig 2, and the solid curve represents the Sensor 1 with a flat bridge/nanopore structure membrane in cell culture solution that has orders of magnitudes higher bipolar peak current density than that of Sensor 2 having negligible peak intensity from 0.8 to -0.8 V without memristor's characteristics as shown in the dotted line. Sensor 1 was able to maintain a reversible membrane potential in an energy favorable manner [17].

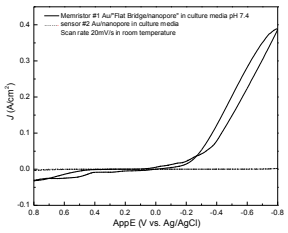

Fig 2. Illustrates Sensor 1 (solid curve)'s CV curve and Sensor 2 (dotted line) at 20mV/s scan rate in cell culture solution.

Evaluation of the Electric Pulse Therapeutic Effectiveness for Cancers

Cyclic Voltammetry (CV) Method. Our prior works revealed Sensor 1 has a special selectivity toward single brain cancer cell over TNA cancer cell, confirmed by CV and CA methods, respectively [8, 18]. Exploring the Sensor 1's therapeutic utility to heal brain cancer was conducted through release energy of the cancer cells using electric pulses as shown in Fig 3A and B. Fig 3B shows the 5 cancer cells/mL's signals were diminished completely after the treatments.

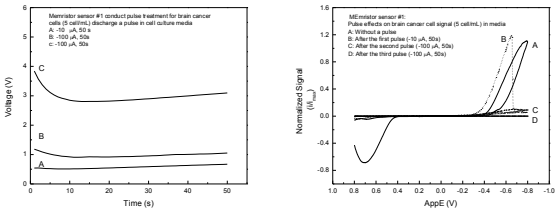

Fig 3 (A) illustrates the three pulses were applied at -10, -100 and -100 µA with 50 s of each; Fig 3(B) illustrates the CV curves after the treatments against control.

Contour Map Multiple Variable Correlation Method (CMMVC). The map shows the gradient of the heat reduced after the 3 pulses discharged in Fig 4 . Our results show the heat reduced by 60, 80 and 100% for sensor #1 after each of the three pulses, respectively compared with Sensor 2 (Fig 5) from our prior work [19] with 50 TNA cancer cell/mL with - 40, 53 and 100% for sensor #2. Each brain cancer cell has a magnitude higher heat released than a TNA cell.

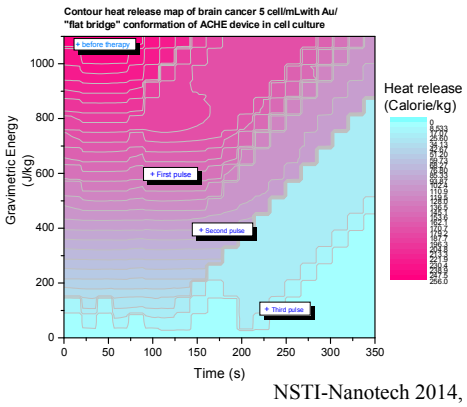

Fig 4 Illustrates Sensor 1's bran cancer heat release map after each of three pulses based on the CV data;.

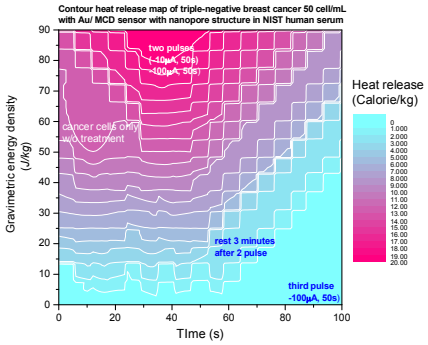

Fig 5 illustrates Sensor 2 detects TNA cancer cells after the three pulses [19].

Non Invasive Monitoring

A non invasive *in vivo* monitoring of a normal breast of a subject became possible using a DSCPO method by applied the three pulses as described above, after each pulse, recorded the DSCPO profile against the control, i.e., without treatment as shown in Fig 6. After three pulses treatments, the symmetry of the peaks was changed compared with that before treatment (solid line), and yet the signal ratio of "action potential" vs. "resting potential" values are in the normal ranges as seen in Fig 7 and there was no heat released. Comparing the mammograms before and after pulse treatments and followed up within 1 year, doctors' writing up as for normal breasts was diagnosed.

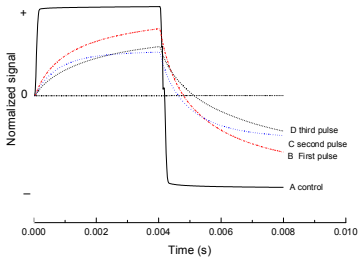

Fig 6 illustrates the DSCPO profiles after each pulse treatment against the control, i.e., before treatment.

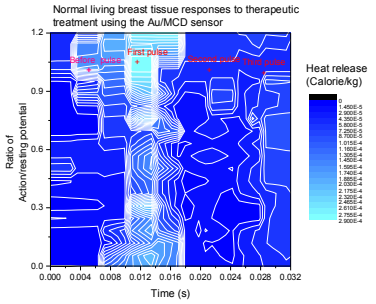

Fig 7 Illustrates the heat release map after three pulse treatments based on the DSCPO method.

CONCLUSION

The results from the preliminary therapeutic function study paved a foundation for further study of the utility of this nanobiomimetic sensor, especially for the applications of the Memristor sensor. The technology offers benefits of simplicity, portability and potentially effectiveness to treat cancer. Study of factors non invasively affects *on in vivo* monitoring is needed in order to pave a road for applying large sample sizes and to gather more information.

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