Organic Nanobiomimetic Memristive/Memcapacitive Devices
Ultrasensitive Direct Detect Matrix Metalloproteinase-2 in Human Serum

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ABSTRACT

Matrix Metalloproteinase-2 (MMP-2) plays a key role in many diseases. A new type of dual-functioning device was developed for fast, direct ultrasensitive detection of MMP-2 using NIST SRM965A reference human serum specimens under label-free, probe-free and reagent-free conditions. The organic memcapacitive/memristive device was based on a self-assembled polymer membrane having polarized 3D array crossing-nanotube structures on a gold substrate that enables the selective inducement of bio-communication with MMP-2. The characteristics of memcapacitor/memristor were observed in the i-V curves of the hysteresis. The Chronoamperometry (CA) method was used with a Detection of Limits (DOL) value of 8.67x10-19 g/mL in PBS solution related to current density between 1.47 µA/cm² and 919.2 mA/cm² over MMP-2 concentration between 20 ag/mL to 100 ng/mL with a Relative Pooled Standard Deviation 1.4% (n=26). The Double Step Chronopotentiometry (DSCPO) method produced a similar impression value of 1.47% (n=18) over MMP-2 concentration 40 ag/mL to 100 ng/mL over energy density between 185 - 0.47 µWHR/cm³. The sensor was able to direct detect MMP-2 in pure NIST serum specimens in the concentrations of 81.15 ± 0.10 ag/mL for normal glycemia, 1.13 ± 0.0016 pg/mL for hypoglycemia and 1.4± 0.001 pg/mL for hyperglycemia serum, respectively.

Keywords: Organic Memristive/Memcapacitive Devices; Nanobiomimetic Polarizable Crossing-Nanotubes; Inducing bio-communication; Direct Electron-relay Transfer System; Energy Storage and Sensing Device; Probe-free, Label-free and reagent-free conditions.

INTRODUCTION

Matrix Metalloproteinase (MMP) is a family of zinc-dependent endopeptidases. The enzymes play a key role in human health for promoting newborn growth, nervous system growth, as well as in promoting various human diseases, such as cancer invasion, osteoarthritis, tissue destruction, diabetes, coronary malfunction, epilepsy and Alzheimer’s [1-4]. MMP’s major role is to degrade the extracellular matrix as a double-edge sword. MMP-2 has been identified as a critical biomarker for diagnosing, monitoring and predicting multiple types of human diseases [4-9]. However, almost 50 MMP inhibitors in clinical trials failed due to lack of specificity of the inhibitor to MMP [10]. Improving sensor performance in the detection of MMPs in a sub fg/mL level among a wide dynamic range implemented with simplified procedures is a paramount challenge in the traditional enzyme-linked immunosorbent assays (ELISAs) method, labeling florescence method and the nanoparticle electrochemical sensing methods [11-12]. This is because most methods are subject to protein interference and time consuming burdensome procedures that hamper reaching the goals. Our prior experiences in the development of nanostructured biomimetic sensors for direct detection of various biological biomarkers have encouraged us to seek an innovative approach and attempt to attack this problem for direct reagent-free detection of MMP-2 [13-18].

Development of polarized microtubules mimicking nature’s microtubules is an increasingly interesting subject in many nanoscale engineering applications [19]. However, utilizing the microtubule mimicking approach to apply to the direct detection of MMP-2 with a reagent-free goal in mind is very challenging, and then the question which follows is how to induce MMP-2 direct biocommunication with the artificial microtubules. Our approach is to build the artificial microtubules with cross-linked organic conductive polymers having multiple chelating imidazole ligands embedded. That enables the polymer ligands to have a strong affinity to coordinate with the zinc ions in the MMP-2. Plus the crossing-bar nanotubes might be favorable in developing a nanostructured memcapacitive/memristive sensor for reagent-free, probe-free direct measurement of MMP-2.

EXPERIMENTAL

Fabrication of the Nanostructured Self-Assembling Membrane (SAM) Gold Memristive/Memcapacitive Chips

The nanostructured biomimetic SAM was freshly prepared by forming cross linked conductive polymers from triacetyl-ß-cyclodextrin (TCD), polyethylene glycol diglycidyl ether (PEG), poly(4-vinylpyridine) (PVP) and bis-substituted dimethyl-ß-cyclodextrin (bM-ß-DMCD) in a self-assembling manner on gold chips with appropriate propositions of the mixture. The polymer mixture was
incubated at 80°C for 2 hrs before injecting it on the chip. After the injection, the chips were incubated for 96 hrs at 37°C, then reincubated again for 2 hrs after washing the chip with high purity water. The procedures of synthesis and characterization of bm-β-DMCD were based on the published literature [20]. MMP-2 enzyme was purchased from Ana Spec (Freemont, CA).

**Characterization of the Biomimetic Microtubule Membrane**

The morphology of the AU/SAM was characterized using an Atomic Force Microscope (AFM) (model Dimension Edge AFM, Bruker, MA). Data collected in TappingMode using silicon probes with 5-10nm tip radius and ~300kHz resonance frequency (Probe mode TESPA-V2, Bruker, MA).

![AFM Image](image)

Fig. 1 depicts the AFM 3D image for device in a small 1 µm length scale. Fig. 2 depicts ordered cross nanotubes in a large 5 µm scale AFM image.

**Evaluation of the Coordination Formation**

Evaluations of the formation of a coordination complex between the MMP-2 and the ligands of the biomimetic membrane were based on a model mechanism proposed in Fig. 3. Two methods were used for the evaluations: (1) A cyclic voltammetry (CV) method was used to compare the dynamic rate constant results of direct electron-relay peaks and the MEM peaks vs. consecutive scan cycles (5) with or without MMP-2 at 200 mV/s scan rate at room temperature at pH 7.4 PBS. MMP-2 concentration is 40 ng/mL. (2) A comparison of Michaelis-menten constant (k_m) results using curves obtained from a chronoamperometric method (CA) described in the following section.

**Quantitation of MMP-2**

Quantitation of MMP-2 was conducted in two methods: the CA method and the Double Step Chronopotentiometry (DSCPO) method. The data were acquired at room temperature under fixed applied potentials for the CA method with 4 MHz data rate in MMP-2 final concentrations ranging from 2.0x10^{-17} g/mL to 1.0x10^{-7} g/mL with triplicates compared with pH 7.4 PBS controls. Curves presented were after taken an absolution for better visualization. Fixed ±10 nA and 4s step time was used with 1 KHz data rate for the DSCPO method with similar MMP-2 concentration ranges with samples run triplicate. MMP-2 samples were freshly prepared. Before the measurements, the standards samples were incubated at 37°C for 2 hours. The preliminary applications were to detect the MMP-2 activities present in the NIST SRM 965A reference human serum samples with known hypo-, normal and hyperglycemia concentrations, respectively. An electrochemical work station was used (Epsilon, BASi, IN) with a software package from BASi. Origin Pro 2016 (Origin Lab Corp., MA) was used for all statistical data analysis and figure plotting.

**MMP-2 Concentration Levels Affect on the Sensor Energy Density Map**

The DSCPO method was used to study the MMP-2 concentration level’s affect on the sensor’s energy density change related to specific capacitance change. The DSCPO results were obtained in the MMP-2 quantitation study described in the above section. The results were based on the equation of volumetric energy density, \( E = C_s \cdot (\Delta V)^2/(2 \times 3600) \), where \( C_s \) is the specific volumetric capacitance, \( C_s = [ -i \cdot \Delta t/\Delta V ]/L \), \( C_s \) is in F/cm³ [21-22]. \( \Delta t \) is the time change in seconds, \( \Delta V \) is the voltage change in V, \( i \) is the current in Amps, and \( L \) is the volume in cm³.

**RESULTS AND DISCUSSIONS**

**Memristive/Memcapacitive Devices Made by Crossing-Bar Polarizable Biomimetic Microtubules**

Figure 1 illustrates the 3D structure of the membrane with an array of vertical nanopillars with crossing-bars in multiple layers in small scale with z value 15.1 nm, \( R_a \) 1.7 nm and \( R_s \) 1.3 nm. The amplitude channel shows changes in slopes and edges of features seen in height image, similar to a derivative of the Height channel. Fig. 2 shows the AFM well-ordered crossing-nanotube image in large scale. The proposed direct electro-transfer (DET) relay mechanism for detecting MMP-2 was depicted in an art model in Fig.3. The right hand side is the simplified MMP model, and the induced direct bio-communication was shown through the zinc ion coordinating side is the simplified MMP model, and the induced direct bio-communication was shown through the zinc ion coordinating geometry, proton and electron transfers and the displacement of water molecules which formed the long electron-relay chain based on a favorable low \( \Delta G \) [22-23]. The advantage of the sensor SAM is that it turns the inhibitory nature of the MMP-2 coordination complex as a potential cancer treatment drug into an agent for stimulating MMP-2 expression and forms polarizable microtubules shown from Fig. 1 to Fig. 3.

![Art Model](image)

Fig. 3. depicts the art model for the proposed polarizable microtubule electron-relay system. The red dot refers to the imidazole receptors in the CD cavity.

**Memristive/Memcapacitive Devices Promote Microtubule Polarizable Behavior**
Comparing the Rate Constant by the CV Method.

Memristor/memcapacitor exhibits not only hysteric charge-voltage and capacitance-voltage curves but also negative and diverging capacitance within certain ranges of the field [25]. Fig. 4(R)’s i-V hysterisis curve demonstrated with a switch point at the origin (0,0) under consecutive scans at 200Hz in PBS solution compared with that of having 40 ng/mL MMP-2 shown in Fig. 4(L). The increased positive and negative nonlinear potential movements of the DET red and DET ox peaks from the origin demonstrate there is a bidirectional polarizable forces exist in the microtubules as the scan cycles increased. Fig. 5A, B and C represent the plots of normalized current of DET red, DET ox and the MEM peaks vs. scan cycles, respectively. Table 1 compares the DET peak and the MEM peak rate constant results vs. scan cycles for with or without MMP-2. The results show the sensor interactions with MMP-2 have drastically broken the balanced, bidirectional, polarizable direct electron-relay and hole hopping, into a more powerful asymmetric system. The rate constant and the amplitude of the MEM peak with MMP-2 increased 123 and 35.6-fold compared with the control indicating electron-activate MMP-2 in order to have a “cysteine switch” made possible which is impossible according to conventional teaching [11-12, 26].

Table 1. Evaluation of the rate constant of monitored normalized peaks signal strength with or without MMP2 vs. scan cycles using the memristor/memcapacitor

<table>
<thead>
<tr>
<th>Type</th>
<th>MMP2 (40 ng/mL)</th>
<th>Rate(^a) (s(^{-1}))</th>
<th>Y0</th>
<th>A1</th>
<th>r</th>
<th>Chi(^2)/DOF</th>
</tr>
</thead>
<tbody>
<tr>
<td>DET red</td>
<td>No</td>
<td>0.019</td>
<td>0.83</td>
<td>0.84</td>
<td>0.998</td>
<td>0.0002</td>
</tr>
<tr>
<td>DET ox</td>
<td>No</td>
<td>0.025</td>
<td>-0.74</td>
<td>0.85</td>
<td>0.986</td>
<td>0.0019</td>
</tr>
<tr>
<td>MEM</td>
<td>No</td>
<td>0.002</td>
<td>-1.44</td>
<td>2.51</td>
<td>0.989</td>
<td>0.0003</td>
</tr>
<tr>
<td>DET red1</td>
<td>Yes</td>
<td>0.04</td>
<td>-</td>
<td>1.02</td>
<td>0.877</td>
<td>-</td>
</tr>
<tr>
<td>DET red2</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DET ox1</td>
<td>Yes</td>
<td>0.008</td>
<td>-1.35</td>
<td>0.955</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DET ox2</td>
<td>Yes</td>
<td>0.045</td>
<td>-0.60</td>
<td>4.50</td>
<td>0.999</td>
<td>0.0036</td>
</tr>
<tr>
<td>MEM</td>
<td>Yes</td>
<td>0.185</td>
<td>1.58</td>
<td>9.3</td>
<td>0.996</td>
<td>0.07</td>
</tr>
</tbody>
</table>

\(^a\)refers to the first order rate constant model for exponential decay of the normalized different types of peaks strength monitored vs. time in second. All other parameters of Y0, A1 are referring to the normalized signal strength per scan cycle. For the control samples, the consecutive 5 scan cycles were at 200mV/s and used to compare with that in the presence of 40 ng/mL MMP2 samples at the same experimental conditions. b For DET red with MMP2, curve fitted with a polynomial model Y = A + B1*X + B2*X^2. c For the DET ox it was fitted with a linear model.

**Comparing the \(K_m\) Constant.** For comparing the \(K_m\) results of the ligands of the sensor membrane affiliated with the MMP-2, Lineweaver-Burke plots were constructed. The \(K_m\) value is 6.75 pM over 7.0x10\(^{-13}\) to 1.4x10\(^{-9}\) M, which is orders of magnitudes stronger complexation than reported MMP-2’s \(K_m\) value for type 1 collagen of 8.5 \(\mu\)M [27-28]. The MMP-2 concentration is between 2x10\(^{-7}\) to 8.0x10\(^{-10}\) M, \(K_m\) value is 1.6x10\(^{-7}\)s and the \(K_m/K_c\) = 6.4x10\(^{13}\) s\(^{-1}\).M\(^{-1}\).

**Quantitation of MMP-2 by the CA Method**

Fig. 6 (L) depicts a plot of current density vs. MMP-2 concentration over the range of 0.02 fg/mL to 100 ng/mL. The CA method has a Detection of Limits (DOL) value of 8.67x10\(^{-18}\)g/mL in PBS solution related to current density between 1.47 \(\mu\)A/cm\(^2\) and 919.2 mA/cm\(^2\) over MMP2 concentration between 20 ag/mL to 100 ng/mL with a

Fig. 4 (L) Illustrates the hysteresis of the i-V curve of the memristor/memcapacitor in 40 ng/mL MMP-2 with consecutive scan cycles at 200Hz scan rate. Fig. 4 (R) depicts the i-V curve in control solution at 200Hz scan rate.
Relative Pooled Standard Deviation 1.4% (n=26). Fig. 6(R) depicts the CA curve profiles from 0.01 ng/mL to 100 ng/mL. Fig. 7(L) depicts the CA curve profiles from 0.02 fg/mL to 40 fg/mL. The insert is the linear regression curve with over the same MMP2 range as in Fig. 7(L). Fig. 7(R) depicts a linear calibration plot of the current density vs. MMP-2 concentration range from 0.01 ng/mL to 100 ng/mL with a linear regression equation \( Y = 33.8 + 8.8X, r=0.999 \) (n=18), P<0.0001, Sy/x=18.6.

Fig. 8(L) depicts the DSCPO voltage curves vs. time at 0.25 Hz at ±10A over 40ag/mL to 100 ng/mL MMP-2 concentrations against the control samples with each sample run triplicates. Fig. 8 (R) depicts the volumetric energy density vs. MMP-2 concentrations

**Direct Measuring MMP-2 in NIST 965A Human Serum Specimens**

The preliminary evaluation of the method application was conducted using the CA method to measure the MMP-2. The sensor was able to directly detect MMP2 in pure NIST serum specimens in the concentrations of 81.15 ± 0.10 ng/mL for normal, 1.13 ± 0.0016 pg/mL for hypoglycemia and 1.4±0.0001 pg/mL in hyperglycemia serum, respectively.

**Energy Density Map with Multiple Variables**

Fig. 9 depicts a 3D map of the relationship between energy density of the sensor, MMP-2 concentration and specific capacitance using the voltage method. It was observed that lower MMP-2 concentration and lower specific capacitance are associated with higher energy density.

**CONCLUSION**

We have demonstrated the advantage of the memristive/memcapacitive device with the biomimetic polarizable microtube membrane that enables direct detection of MMP-2 with ag/mL level sensitivity in human serum specimens and the DOL reached orders of magnitude lower than published reports under antibody-free, tracer-free, and reagent-free conditions with simplified procedures by two instrumental methods. The results show a feasible application for the development of commercial fast and real-time monitoring of MMPs devices for various diseases.

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**REFERENCES**

See www.abs-isensors.com