Electrochemical Study of Sensor with Aptamer Specific to Glioblastoma

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***Abstract* – An aptasensor consisting of a gold disc electrode and aptamer Gli-233 specific to glioblastoma was prepared with different methods and studied by electrochemical and microscopic methods. It was found, that the real electro active surface area and electric double layer capacitance changed with the electrode modification. The highest capacitance was registered for the electrode modified with the simultaneous immobilization of Gli-233 and additional blocking agent (mercaptoethanol). It was shown that to develop an impedimetric sensor on the basis of Gli-233, it is necessary to optimize the conditions for the signal registration.**

***Index Terms* – Aptamer, electrochemical sensor, glioblastoma.**

I. INTRODUCTION

GLIOBLASTOMA IS A TUMOR developing from star-shaped glial cells that support the health of the nerve cells in the brain. It is the most common cancerous primary brain tumor in adults. Glioblastoma (or glioblastoma multiforme (GMB)) is diffuse having threadlike tendrils that extend into other parts of the brain. That is, the glioblastoma is fast growing and likely to spread [1]. Diagnostics is based on computed tomography (CT) and magnetic resonance imaging (MRI) [2]. A surgical biopsy is used as a last resort to confirm the diagnosis. In this procedure, a sample of abnormal cells is extracted and tested in a pathology laboratory. Cell necrosis characteristic of GBM is the main factor for being glioblastoma.

A surgical biopsy is a quite traumatic method. The brain biopsy can result in brain swelling or bleeding. It can also lead to infection, seizure, stroke, and coma. Also from time to time, the tests on the taken tissue sample are inconclusive. This requires the procedure repetition. That is why, a developing new glioblastoma diagnostic methods is an extremely important task. Methods based on tumor markers' detection in blood attract much attention recently [3,4]. In this regard, aptasensors–biosensors based on aptamers–are developing intensively [5,6].

Aptamers are oligonucleotides or peptides that are able to bind target molecules. The most important advantages of aptamers are high specificity to the target molecules, chemical way of their synthesis, and stability higher than of the other biomedical objects. Aptasensors with different signal registration principals are used. According to Rapini with co-author [7], more than a half of aptasensors developed are electrochemical. Along with the high sensitivity, electrochemical aptasensors are easy to use and rather cost-effective.

II. PROBLEM STATEMENT

A DNA-aptamer specific to glioblastoma was developed and tested in Krasnoyarsk State Medical University. It showed good specificity and affinity for glioblastoma cells derived from postoperative tissues (non-published data).

As it was shown by us earlier, aptamer's electrochemical behavior can differ from the predicted or common [8] that would change the principal of the aptasensor operation. That is why, before the aptasensor construction and analytical testing (with the target molecules), electrochemical properties of the aptamer have to be studied. In the present work, DNA-aptamer Gli-233 specific to GMB was bind to a golden electrode surface and studied. The aim was to find whether the Au/Gli-233 system may be potentially used for electrochemical sensing.

III. EXPERIMANT DETAILS

Electrochemical station CHI-660 (CH Instruments, USA) was used for the electrochemical data registration. Background electrolyte was phosphate buffer solution (PBS), pH=7.4. Redox probe 0.025 M equimolar $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ solution was used. Degassing for 30 min with Ar was carried out before each experiment. Electrochemical three-electrode cell consisted of a working (indicator) electrode – Au disc, d=2 mm; a counter (auxiliary) electrode – Pt wire; and a reference electrode – Ag/AgCl (with 1 M KCl). Confocal microscopic data (CLSM) was obtained using confocal laser scanning microscope LSM 780 NLO (Carl Zeiss instrument, Germany).

IV. RESULTS AND DISCUSSION

Electrochemical aptasensors consisted of an electrode (often it is golden, but carbon and others are used as well) covered with the layer of an aptamer and additional blocking agent – mercaptohexanol or mercaptoethanol.

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Mercaptoethanol (MET) is used to fulfill the aptamer layer defects. According to a common idea, aptamer layer covers the electrode surface and isolates it from the redox probe, hindering it to react and give the signal – current peak. Ideally, MET fills the defects of this layer improving the signal blanking.

To study the influence of the aptamer presence on the electrode surface, the following experimental scheme was applied (Fig. 1). First of all, Au electrodes were cleaned with a very aggressive Piranha solution (sulfur acid with hydrogen peroxide mixture). To modify the electrode with aptamer, or MET, it was covered with the modifier solution and kept for 20 h at 4°C and 100% humidity for immobilization occur. Then, the modifier was deleted with the PBS solution. Modified electrodes were always kept under the PBS drop, in order to prevent the modifier's layer to dry and destruct.

In this work we study and compare (Fig. 1): bare Au disc electrode (Au); Au electrode with MET layer (Au/MET); gold electrode with aptamer layer (Au/Gli-233); Au electrode cover with aptamer and MET consistently (Au/Gli-233/MET) and simultaneously (Au.Gli-233+MET).

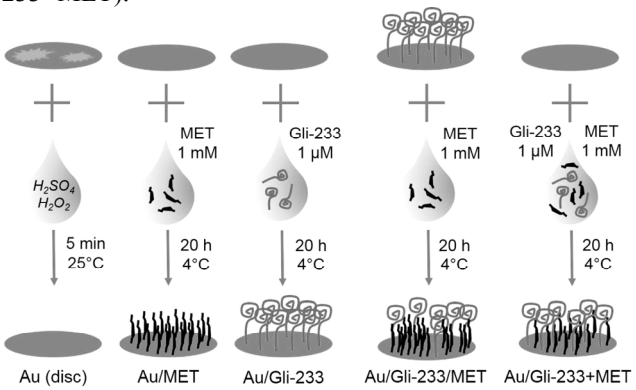

Fig. 1. Electrodes preparation scheme and labeling.

As prepared electrodes were immersed into the closed cell with the degassed solution and electrochemical data was obtained. First of all, cyclic voltammograms (CVs) were recorded in redox probe solution (Fig. 2) to determine the real electro active surface area of the electrodes (S_{real}) via Randles-Shevchik equation. Then, CVs in PBS (not shown) were also obtained to find out the EDL capacitance (C_{dl}) of the electrodes via curves integration and area calculation.

The calculated values of S_{real} and C_{dl} are shown in Table I. It can be seen, that both values obtained for bare Au disc electrode have a large error. This might be connected with the bare electrode surface roughness. It seems as if aptamer or MET presence on the disc electrode led to the roughness lowering. This is possible when the modifier fills the Au surface defects, which was approved by the CLSM data (Fig. 3). It is seen, that aptamer molecules located predominantly on the "scratches" of Au electrode surface. Actually, this is not the ideal situation for the aptasensor. The aptamer layer is believed to cover the electrode in the form of a monolayer. But, there is not any approval in the literature that the formation of such a monolayer has been achieved. So, the majority of the aptasensors still work giving the signal, even without monolayer coverage.

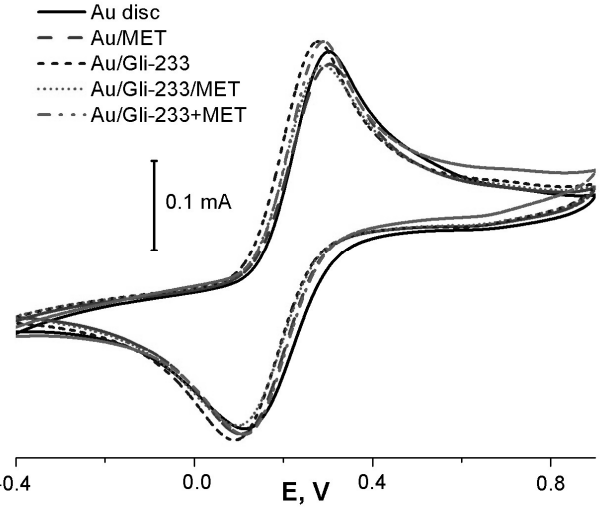

Fig. 2. CVs obtained in equimolar 0.025 M [Fe(CN)6]3-/[Fe(CN)6]4- solution in PBS for electrodes: (solid line) Au disc; (dashed line) Au/MET; (short dashed line) Au/Gli-233; (short dotted line) Au/Gli-233/MET; (dash-dotted line) Au/Gli-233+MET.

TABLE I
THE VALUES OF S_{REAL} AND C_{DL} CALCULATED FROM THE CV DATA

Electrodes	S_{real}, cm^2	C_{dl}, F
Au	$(4.2\pm0.9)\times10^{-2}$	$(6.8\pm0.9)\times10^{-5}$
Au/MET	$(4.1\pm0.2)\times10^{-2}$	$(5.9\pm0.6)\times10^{-5}$
Au/Gli-233	$(4.6\pm0.3)\times10^{-2}$	$(6\pm1)\times10^{-5}$
Au/Gli-233/MET	$(3.7\pm0.8)\times10^{-2}$	$(5.7\pm0.7)\times10^{-5}$
Au/Gli-233+MET	$(4.4\pm0.3)\times10^{-2}$	$(1.4\pm0.6)\times10^{-4}$

So, it was decided not to take the bare electrode into account and compare the data for modified electrodes only inside their group. Next, it should be noted, that Au/Gli-233/MET electrode has large error for S_{real} value as well. This electrode was exposed to the immobilization twice as longer than the other electrodes (40 h that is 20 h for Gli-233 and additionally 20 h for MET).

It is known, that DNA molecules on the gold surface are able to migrate. So, it can be assumed, that additional 20 h treatment with MET led to the aptamer layer reconstruction. This reconstruction may be affected with unaccounted conditions, so it resulted in the experiment repeatability decreasing.

Au/Gli-233 and Au/Gli-233+MET electrodes exhibit almost the same S_{real} values. The question is whether MET affects the surface somehow? Au/MET real surface area is one of the smallest in this group. So, we suppose MET might does not fill the aptamer layer defects.

However, the EDL capacitance values made us reconsider this point. Here, the electrode without MET has

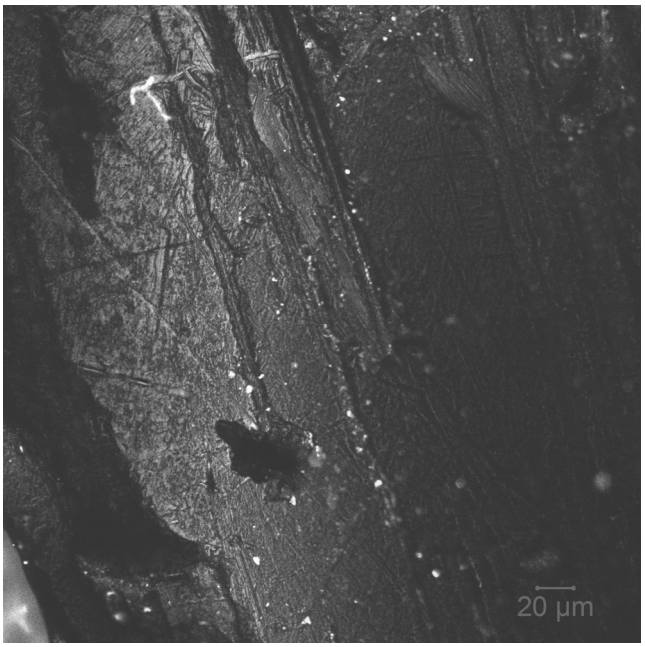

Fig. 3. CLSM image for the Au/Gli-233 electrode surface (bright spots are for the aptamer).

demonstrated the largest error, and Au/Gli-233+MET exhibited the highest C_{dl} value. So, we supposed that the MET presence at the stage of aptamer layer formation influenced its structure providing better EDL capacitance on the electrode/solution interface.

Thus, the electrode prepared via Au/Gli-233+MET scheme may be potentially used for a capacitive aptasensing of the glioblastoma tumor markers. To learn more on this issue, electrochemical impedance spectroscopy (EIS) experiments were carried out. The Nyquist plots obtained for the electrodes under study in PBS are presented in Fig. 4, and the complex capacitance plots calculated from them are in Fig. 5 (calculation method for the case of electrochemical biosensors with modifiers layers is described in literature [9]).

These conditions correspond to a non-Faradic electrochemical process. Here, the double layer capacitance is graphically found as the amplitude of the semicircle part in the plot of the complex capacitance (Fig. 5). In the case under study, there are no distinct semicircles in the plots. But additional modeling (not shown) allowed estimation of such C_{dl} values giving quite close values for modified electrodes (around $(1.3…1.8)\times10^{-5}$ F.

So, the two techniques – CV and EIS – in case of non-Faradic processes (when the data recording carried out in the background electrolyte without any Faradic processes on the electrode surface) provide quite a different data. We suppose, this may be connected with the time necessary for an equilibrium state to be achieved. EIS requires longer time for the system to get ready for the measurements (from hours to days in some cases [10]). Thus, the optimal conditions for the EIS data recording are need to be found.

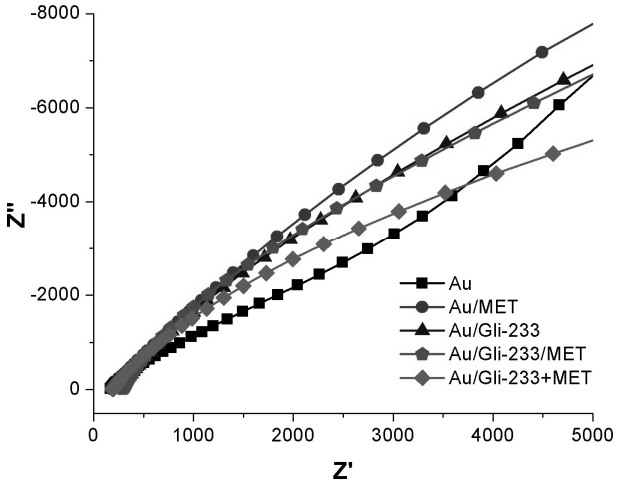

Fig. 4. EIS obtained in the background electrolyte for electrodes: (square mark) Au disc; (circle mark) Au/MET; (triangle mark) Au/Gli-233; (pentagon mark) Au/Gli-233/MET; (rhombic mark) Au/Gli-233+MET.

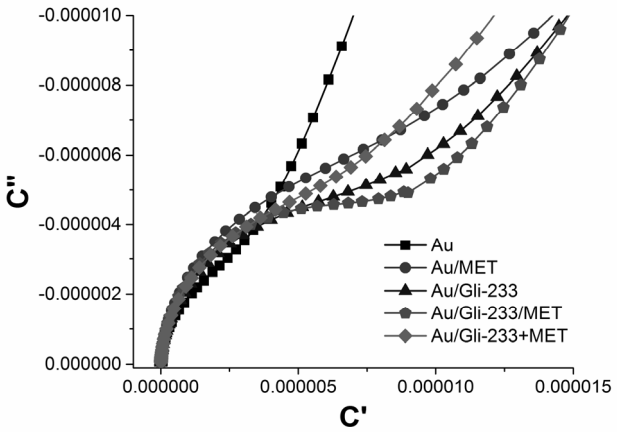

Fig. 5. Complex capacitance plots constructed from the EIS data (Fig. 3): (square mark) Au disc; (circle mark) Au/MET; (triangle mark) Au/Gli-233; (pentagon mark) Au/Gli-233/MET; (rhombic mark) Au/Gli-233+MET.

V. CONCLUSION

In the present work, the system of Au electrode and aptamer specific to glioblastoma was studied. It was shown, that the repeatability of the experiments increases after bare Au electrode modification that may be connected with the surface defects filling with the modifier(s). It was also found that the electrode modification method with the simultaneous Gli-233 and MET immobilization may lead to the better surface layer formation. Moreover, it was established, that CV and EIS data on the EDL capacity differs, so the EIS experiment conditions require further optimization.

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