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Visualization of Nanoconstructions with DNA-Aptamers for Targeted Molecules Binding on the Surface of Screen-Printed Electrodes

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ABSTRACT

Nanoconstructions of gold nanoparticles (NPs) obtained via pulsed laser ablation in liquid with DNA-aptamer specific to protein tumor marker were visualized on the surface of screen-printed electrode using scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). AuNPs/aptamer nanoconstuctions distribution on the solid surface was studied. More uniform coverage of the carbon electrode surface with the nanoconstuctions was showed in comparison with DNA-aptamer alone on the golden electrode surface. Targeted binding of the tumor marker molecules with the AuNPs/DNA-aptamer nanoconstuctions was approved.

Keywords: nanoconstruction, targeted binding, confocal laser scanning microscopy, aptamer, aptasensor, fluorescence, screen-printed electrode.

1. INTRODUCTION

Aptasensors are biological sensors based on aptamers that are able to specific bind of targeted molecules.[1] These sensors appeared in the very beginning of this century exhibit a variety advantages in comparison with immunosensors and enzyme-based sensors, such as great stability, extreme sensitivity and low detection limit.[2] Depending on the signal formation strategy, the aptasensors can be optical, fluorescent, electrochemical, etc.[3]

Electrochemical aptasensors consist of solid electrode, usually golden or carbon, and aptamers layer on it.[4] The most usual targeted molecules for aptamers specific binding are proteins (including, tumor markers, etc.), pesticides, toxins, drugs, etc. However, this could be whole cells, or tissues, as well. Aptamers targeted bind the required molecules, and electrochemical signal is measured. In this regard, aptamers localization and distribution onto the electrode surface are crucially important factors for the aptasensor functioning in the whole. Nevertheless, to the present days, effective and reliable techniques for these important factors estimation were not proposed.

Advances in optical technologies, precise mechanics, computer control technologies, and image processing, as well as the development of fluorescent approaches in conventional microscopy, have significantly enhanced the capabilities of optical microscopy, and make it a unique tool for research and diagnostics. The confocal laser scanning microscopy method now seems to be one of the best achievements of light microscopy. It is successfully applied for the microscopic study of the biological objects, and organic molecules that exhibit auto-fluorescence or stained with the fluorescent labels. The operating wavelength of the detected fluorescence for this method is in 400-800 nm range.

In the present work, the results of a study the localization and distribution of biological objects–DNA-aptamers, specific to tumor markers for lung cancer, on the surface of screen-printed gold and graphite electrodes are presented.

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2. EXPERIMENT DETAILS

2.1 Materials

DNA-aptamers specific to lung cancer were previously selected[5] and received from IDT technologies, USA. Au NPs were obtained via pulsed laser ablation in liquid as described in previous work.[6] Golden screen-printed electrodes (Au SPE) were manufactured in "Electroconnect", ltd. (Novosibirsk, Russia). Carbon screen-printed electrodes (CSPE) were received from RUSens (Moscow, Russia). Chemicals (phosphate buffer solution, Au electrode pretreatment solution, etc.) of analytical grade were obtained from Sigma Aldrich, and were used as received without additional purifications.

Blood plasma of lung cancer patients was taken from patients who had undergone complete, curative resection of their tumors before the surgery. Informed consent was secured from the patients. Experimental protocol used was approved by the Local Committee on Ethics of the Krasnoyarsk Regional Clinical Cancer Center named after A.I. Kryzhanovsky and Krasnoyarsk State Medical University, Krasnoyarsk, Russia.

2.2 Methods

Aptamers immobilization on Au SPE was carried out through the procedure described in previous work.[7] Since aptamers are immobilized via thiol-groups binding to gold, Au NPs were used to bind them with the surface of CSPE. In this case, aptamers colloid was mixed with Au NPs water colloid in the concentration ratio of 1:10. Than the colloidobtained was incubated at 4°C for 12 h. CSPE surface without special pretreatment was covered with AuNPs/aptamers using micropipette. All the modified electrodes were kept under buffer solution in wet air atmosphere (Petri dish) at 4°C. Blood plasma was incubated according to the procedure previously described.[7]

Scanning electron microscopy (Vega 3 SBH, Tescan, Czech Republic) was used for electrodes surface morphology study before and after aptamers immobilization. Fluorescent microscopic images of the modified electrodes surface were obtained using Confocal laser scanning microscope LSM 780 NLO Carl Zeiss, Germany. Objectives of 20×, 100×(oil) were used. CLSM 3D images were obtained in a z-stag mode with 3D reconstruction.

3. RESULTS AND DISCUSSION

SEM-images of both golden and carbon electrodes after modification are presented on Figure 1. It can be seen that after vacuum setting that is necessary for SEM-analysis, aptamer molecules on the electrodes surface seems to agglomerate, and the layer loses its structure. Aptamers show globular shape instead of chain one. Thus, SEM is not able to provide somehow reliable information on the aptamers localization and distribution.

CLSM was used to reconstruct the real picture of aptamers and AuNPs/aptamers nanoconstructions layer structure on the solid surfaces when it is covered with buffer solution. To obtain images the fluorescent conditions for the samples under study were established using aptamer colloid and blood plasma without immobilization. Excitation wavelengths of aptamer and blood plasma protein were found to be 405 and 561 nm, respectively. Their fluorescence maxima were placed in the regions of 450-470 nm for aptamer, and 610-630 nm for plasma protein. Choosing one of these spectral regions of registration it is possible to visualize aptamers and blood plasma protein on the surface simultaneously or separately.

Figure 2 demonstrates 3D images of aptamer and AuNPs/aptamer distribution on Au SPE and CSPE, respectively. Aptamer molecules were colored in green. Gold nanoparticles do not show fluorescence activity and were not visualized. One can see that in the case of carbon electrode and AuNPs/aptamer nanoconstruction, the distribution of the biological component on the solid surface is more uniform than that of aptamers layer on Au SPE. However, it is impossible to estimate the shape or to establish the certain conformation of the aptamer molecules. It is still possible that they change their initial localization or conformation during the analysis procedure.

To prove the aptamers conformation stability we checked their targeted binding ability to the blood plasma protein. Golden electrodes modified with aptamers, and carbon electrodes modified with AuNPs/aptamer nanoconstructions, were incubated under blood plasma, rinsed, and covered with drop of phosphate buffer. The results were similar for both systems. Figure 3 represents images obtained by CLSM for golden electrode modified with aptamer binded to tumor marker protein from the blood plasma sample.

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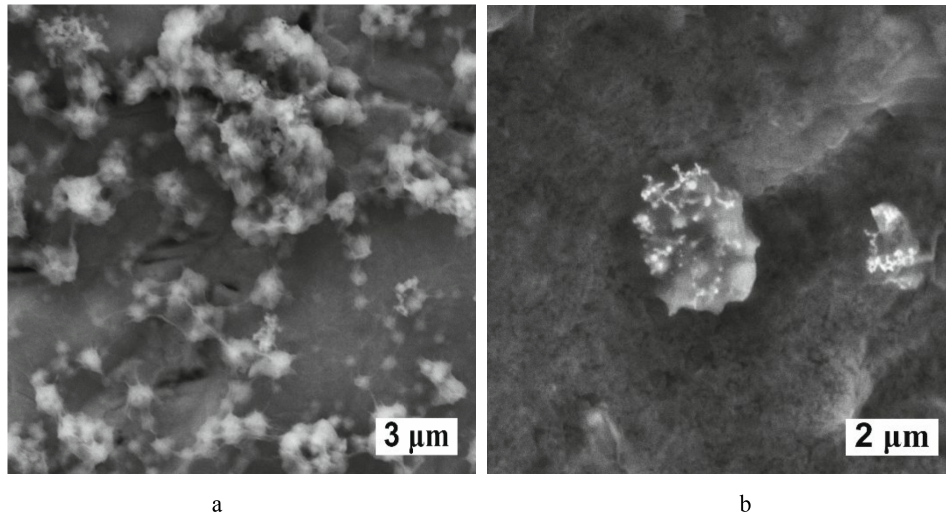

Figure 1. SEM-images obtained in the mode of secondary electrons detection for the systems aptamer@Au SPE (a) and AuNPs/aptamer@CSPE (b).

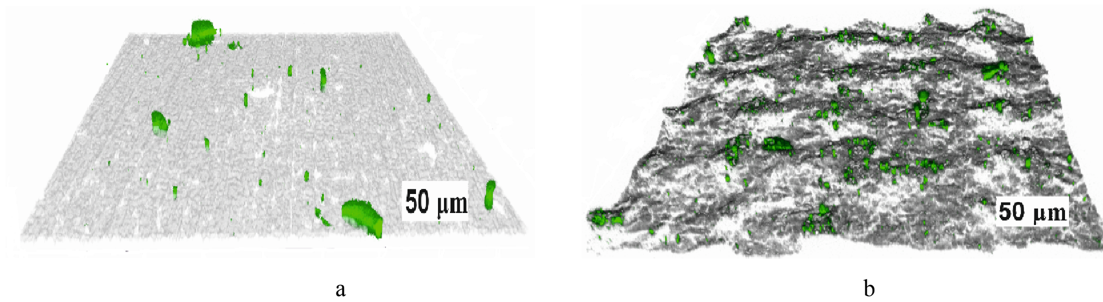

Figure 2. CLSM 3D images obtained for the systems aptamer@Au SPE (a) and AuNPs/aptamer@CSPE (b) covered with phosphate buffer solution. Aptamers are colored in green.

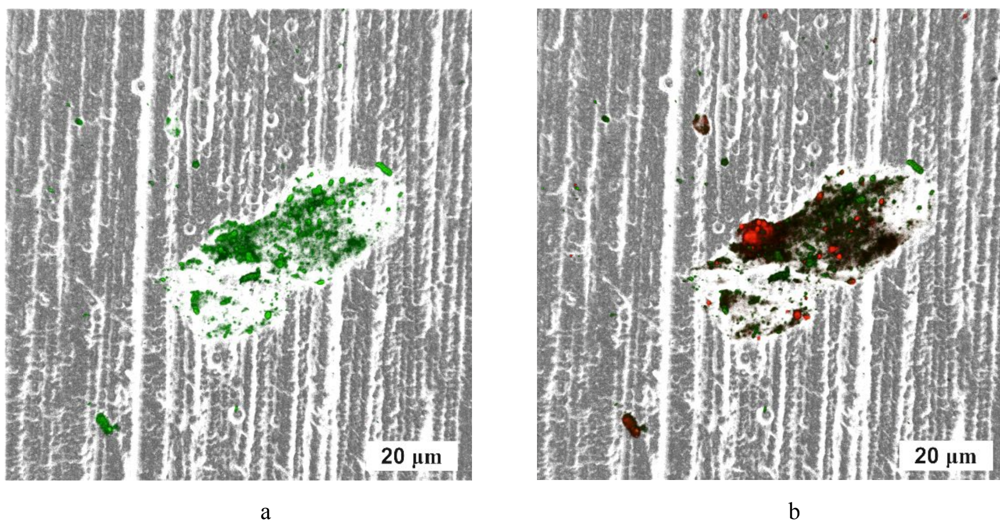

Figure 3. CLSM images obtained for AuNPs/aptamer@CSPE covered with phosphate buffer solution in the mode of aptamer (a) and aptamer+protein (b) fluorescence recording. Aptamers are colored in green, blood plasma protein–in red.

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It is seen, that blood plasma protein (red spots), which should be specific binded with the aptamer, localized on the top of green spots–aptamer molecules. There are no red spots on the electrode surface alone can be seen. It proves the fact that protein is binded through the targeted specific process. Moreover, aptamers lose the ability to bind target molecules when their conformation is changed. So to say, the working conformation of aptamers was not destructed during immobilization method and the analysis procedure. Thus, it is quite likely that CLSM method allows visualizing the original localization and distribution aptamers and nanoconstructions with aptamers on the electrodes surface. The results obtained are important for the further research work on the development of a complex approach to the estimation of a quality of a modified aptamer layer on the electrodes surface.

4. CONCLUSION

Confocal laser scanning microscopy was applied to visualize DNA-aptamers on the surface of screen-printed electrode. The imaging was performed in water solution that is normal condition for aptasensor operating. It was found, that nanoconstructions of aptamers with gold nanoparticles is uniformly distributed over the carbon SPE. In the case of aptamers alone on the golden electrode, the distribution was more of island-type.

The stability of aptamer working conformation to the immobilization and analytical procedures was shown. Aptamers kept their ability to targeted bind blood plasma protein. The localization of protein coincided to that of aptamer molecules.

It was concluded, that quite likely confocal laser scanning microscopy method allows visualizing the original localization and distribution of aptamers and nanoconstructions with aptamers on the electrodes surface. Thus, this method can be successfully applied to such systems investigation. This technique will became an important part of the complex approach to the estimation of a quality of a modified aptamer layer on the electrodes surface that is our next aim.

5. ACKNOWLEDGEMENTS

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