IMMUNOGLOBULIN G ADSORPTION AND REACTIVITY ON ZnO NANOSTRUCTURES INVESTIGATED BY MEANS OF CONFOCAL FLUORESCENCE

J. Ogorodniichuk[1], R. Sonko[1], J.-M. Janot[2], M. Bechelany[2], R. Viter[3], N. Starodub[1]
[1]*National University of Life and Environmental Sciences of Ukraine*
Heroyiv Oborony st., 15, Kyiv 03041, Ukraine, ogorodniichuk@mail.ru
[2]*Institut Européen des Membranes, Université Montpellier 2*
Place Eugene Bataillon, 34095 Montpellier Cedex 5, France
[3]*Odessa National I.I. Mechnikov University*
Dvoryanska st., 2, Odessa 65026, Ukraine

Antibodies, due to their high antigen specificity and affinity, provide excellent probes for recognizing and detecting wide range of substances. The development of such bioanalytical systems requires antibody immobilization on various solid supports with the least impact on their structure at the same time. Another significant factor, which has place in biosensorics and rapid sensitive bioassay methods, is proper orientation of antibodies on surfaces [0].In our study, we are working out rapid and high specific method for substances determination using antigen-antibody specific reaction on ZnO nanostructures cowered surface by means of confocal fluorescence method.

During experiment, several kinds of surfaces such as glass cleaned with ozone, hydrophilic glass with ZnO nanostructures on the surface and glass with ZnO nanostructures on the surface functionalized with Hexamethyldisilazane (HMDS) as a self-assemble monolayer (using vapor coating technique) for protein interaction with CH_3 groups were tested to define which one provides the best protein saturation and most stable protein adsorption. As working solutions such reagents were used: specific couple of Standard Human Serum (Ag) and Swine Anti Human IgG (Ab), solution of Avidin, solutions of Ag, Ab, Avidin labeled with Alexa (Alexa Fluor® 594 carboxylic acid, succinimidyl ester).

First, it was defined that after procedure of proteins labeling with Alexa their properties to react with surface or provide complex Ag-Ab do not change.

After testing surfaces with different properties listed above it was determined that the most appropriate surface was glass covered with ZnO and HMDS with hydrophobic surface. It provided stable layer of protein on the surface while it was observed proteins desorption and uneven coverage on the other surfaces. In addition, it was found surface saturation points for Ag and Ab which where ~115 ng/cm^2 and ~160 ng/cm^2 respectively. Such values provide an opportunity to make some estimations about Ab orientation on the surface [2].

After providing specific immunoreaction on ZnO-HMDS hydrophobic surface following algorithm Ab-PBS-Ag-PBS it was observed that about 50% of Ab bound with the surface reacted with Ag.

After surface saturation with Ab it was still observed clear space on the surface, where Avidin molecules had been able to immobilize. It can be explained with Ab orientation and size of Ab molecules (~160 kDa) that is a few times bigger than Avidin (66-69 kDa) but very close to Ag (67 kDa). Therefore, it was concluded it is necessary to block uncovered spaces on the surface to prevent nonspecific Ag binding.

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[2] Meredith E. Wiseman and Curtis W. Frank, Antibody Adsorption and Orientation on Hydrophobic Surfaces, Langmuir 2012, 28, 1765−1774.