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Utilization of antibody-nanoparticle conjugates as a tool for immunochemistry with ICP-MS detection

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Abstract: Immunoanalytical techniques are key methods of application in clinical diagnostics, genomics, proteomics and other biochemical and molecular biology disciplines. Most often, they are based on the ability of labeled antibodies to bind specific antigens. It is possible to use a large variety of nanomaterials that are designed, synthesized and adapted to allow highly sensitive detection of advanced immunoassays. Detection can be a highly efficient analytical method of laser ablation followed by inductively coupled plasma mass spectrometry (LA-ICP-MS), which allows the detection of elemental tags suitably conjugated to antibodies. The aim of this work was to conjugate model anti-mouse antibody on a surface of 10nm and 60nm gold nanoparticles and choose the better one for conjugation experimentally by using dot-blot immunobinding assay followed by LA-ICP-MS. It has been experimentally proven that 10nm gold nanoparticles are more suitable for conjugation with antibodies because of lower non-specific sorption on a membrane.

Key Words: Immunochemistry, dot-blot, gold nanoparticles, LA-ICP-MS

INTRODUCTION

A nanoparticle is an object with at least one of three dimensions smaller than 100 nm. The properties of nanoparticles allow their application not only in material engineering, chemical synthesis, semiconductor technologies, etc. but also in biochemistry, molecular biology, biomolecular engineering and biomedicine. They are used in *in vitro* and *in vivo* imaging, as well as in biochemical analyses (Filipponi and Sutherland 2013). Their properties and ability of biomolecules conjugation enable modifications of traditional immunoassays (Hu and Li 2011).

Immunoassays are bioanalytical techniques using antibodies for a specific detection and quantification/quantitation of target molecules by unique antigen-antibody reactions. These methods find abundant utilization especially in the laboratories of clinical chemistry and biochemistry. They are used to detect and quantify low amount of proteins, hormones, various metabolites and pathogens, drugs, and even nucleic acids in a sample. They are highly specific and sensitive and allow qualitative and quantitative detection of the analyte in a complex medium, such as urine, serum, whole blood or tissue, without necessary previous extraction (Wu 2017).

By conjugating of the antibodies on the surface of nanoparticles, it is possible to achieve better detection limits of immunochemical methods. In addition, a wide range of nanoparticle properties make it possible to use different analytical methods for detection: colorimetric, electrochemical, or optical (fluorescence, chemiluminescence). This often allows a user to select nanoparticles according to the laboratory equipment without the need to purchase new costly detection devices (Cardoso et al. 2012).

Indirect quantification of antigen by labelled antibody is increasingly used for laser ablation followed by inductively coupled plasma mass spectrometry (LA-ICP-MS). The most important advantage of mass spectrometry is the ability to simultaneously measure several elements in one measurement and its sensitivity makes it possible to detect the presence of the ultra-trace element in almost any matrix (Waentig et al. 2012).

MATERIAL AND METHODS

Materials

All chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) in ACS purity, unless otherwise indicated. All solutions were diluted using ultra-pure Mili-Q water prepared by a Milipore purification system (Bedford, MA, USA).

Mouse Immunoglobulin Natural Mouse IgG protein ab198772 (Abcam, Cambridge, United Kingdom) was used as the model antigen, and the anti-mouse IgG HbL Ab6708 mouse antibody (Abcam, Cambridge, UK) was used as the model antibody.

For the preparation of conjugates, the commercial kits of gold nanoparticles GOLD Conjugation Kit (10 nm, 20 OD) ab201808 (Abcam, Cambridge, UK) and GOLD Conjugation Kit (60 nm, 20OD) ab188216 (Abcam, Cambridge, UK) were used.

Preparation of conjugates

The conjugation was performed according to the manufacturer's instructions. Briefly, the diluted antibody by antibody diluent was mixed with the reaction buffer and subsequently lyophilized nanoparticles. A few minutes later (depends on a size of nanoparticles) a Quencher was added.

Dot-blot

Before use, the PVDF membrane (Bio-Rad, USA) needs to be activated by soaking in methanol and in blotting buffer (50% (v/v) 2x blotting buffer with 40% (v/v) H_2O and 10% (v/v) MetOH needs to be always newly prepared) (2x blotting buffer 25mM Trizma base, 150mM glycine, 10% (v/v) methanol) both for 30 s. Then the membrane was placed on a filter-paper wetted by blotting buffer to prevent drying. Further, immunoglobulin samples (0.5 µl) were applied and dried for 20-30 minutes at a laboratory temperature. All following steps were carried out at room temperature, 60 rpm using Multi RS-60 (Biosan, Latvia). Next step was blocking of the membrane by 10% skimmed milk in PBS (137mM NaCl, 2.7mM KCl, 1.8mM KaH_2PO_4, and 10mM Na_2HPO_4, pH 7.4) for 30 minutes. Subsequently, incubation with antibody in PBS with 1 mg/ml BSA was carried out for 1 hour and following by washing. After the three times repeated washing with PBS containing 0.05% (v/v) Tween-20 for 5 min, the membrane was analysed by LA-ICP-MS.

LA-ICP-MS

LA-ICP-MS analyses of the dot blots were performed as described in Tvrdonova et al. (2018). A laser ablation system UP213 (NewWave, USA) emitting laser radiation of 213 nm with a pulse width of 4.2 ns and a quadrupole ICP-MS spectrometer Agilent 7500ce (Agilent Technologies, Japan) was used. The imaging of the dot blots was performed using the following ablation parameters: a laser beam diameter of 110 µm, laser beam fluence of 2.5 J/cm^2, a repetition rate of 10 Hz, a scan speed rate of 150 µm/s, and a distance between individual lines of 115 µm. The whole spot was ablated and the Au signals were measured. The sum of intensities across the whole spot was then calculated. The images of the dot blots were created using lab-made software Laser Ablation Tool for processing of raw data and Excel for presenting the maps as surface plots with the intensity shown in a color-coded intensity scale.

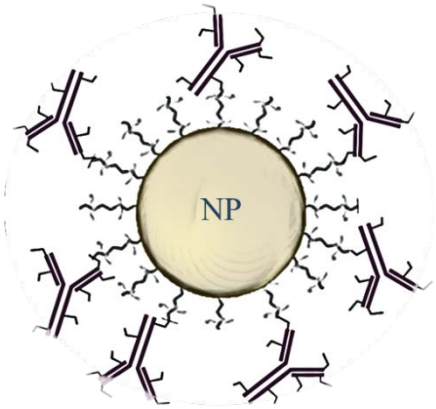
RESULTS AND DISCUSSION

Preparing of conjugates

Two types of commercial nanoparticles were used to prepare conjugates. The nanoparticles were delivered as a lyophilized mixture and the conjugation reaction was initiated by the addition of the antibody and the reaction buffer that was part of the kit. Immobilization of antibodies to the functional

surface of nanoparticles was performed by covalent bonding through primary amines. In the case of antibodies, these are lysine residues.

Figure 1 Scheme of random spatial orientation of antibodies on the functionalized surface of NPs bound by lysine residues according to Richards et al. (2017)

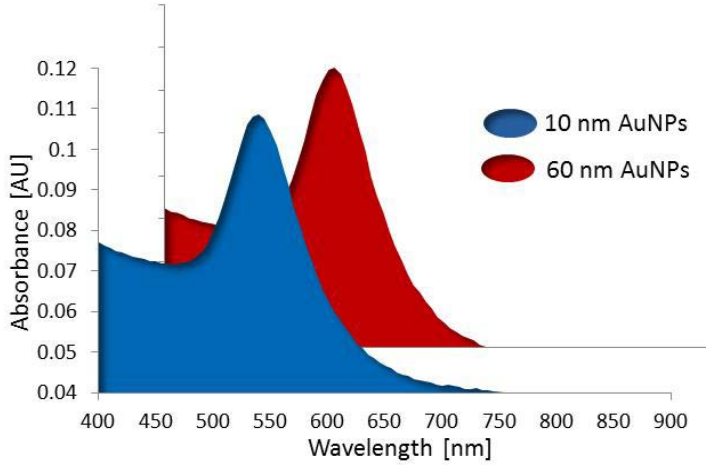


Covalent antibody binding to the modified surface of the gold nanoparticles traditionally occurs *via* the functional groups contained in the side chains of the amino acids forming the primary structure of the antibody. The resulting orientation of the bound antibodies to the particle is random (Figure 1). This is because the primary structure of the antibody always contains a greater number of binding amino acids and the interaction can occur with any one of them (Richards et al. 2017).

Analysis of dot-blot membranes by LA-ICP-MS

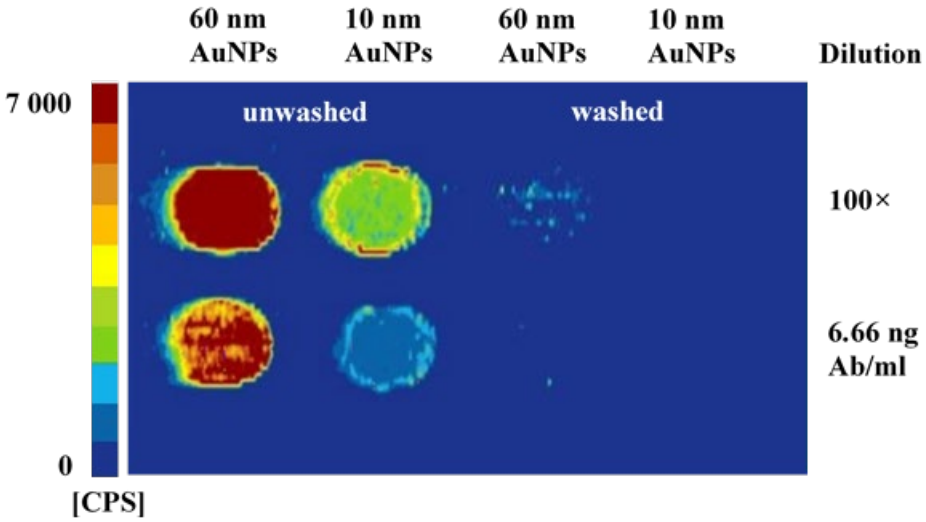
At first, the suspensions of nanoparticles were adjusted to the same optical density (OD). Absorbance spectra of conjugated nanoparticles were obtained by spectrophotometric analysis. The absorbance spectra are shown in Figure 2.

Figure 2 Absorbance spectra of 10nm and 60nm gold nanoparticles



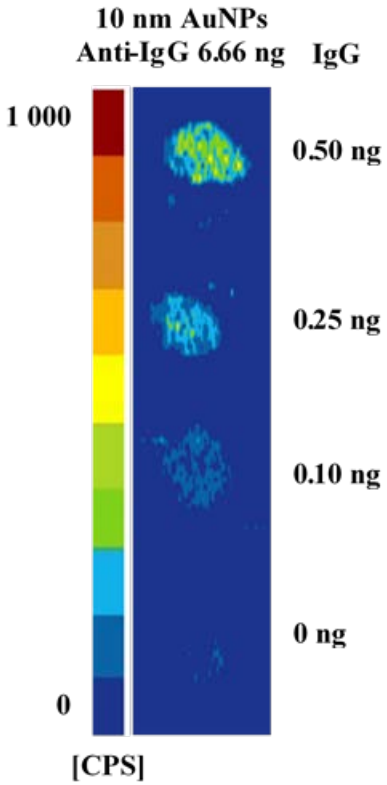
Nanoparticles of the same OD were diluted 100× and to the concentration of antibody of 6.7 ng/ml. 0.5 µl of these suspensions were applied on the activated PVDF membrane (without presence of antigen). In one case, the spot was analysed by LA-ICP-MS. In the second case, the membrane was washed before analysis to demonstrate the non-specific sorption on the membrane without the antigen. As seen from Figure 3, 60nm nanoparticles provided higher signal despite the same OD (in case of no washing). It is caused by higher number of atoms forming the 60nm nanoparticle and therefore, higher Au signal was observed. Negative side effect of 60nm nanoparticles was higher nonspecific sorption on the membrane (when the membrane was washed). As a result, 10nm particles were chosen as more suitable for conjugation.

Figure 3 Nonspecific sorption evaluations



The ability of antibodies to bind the antigen was verified by dot-blot technique followed by LA-ICP-MS, (Figure 4). The intensity of Au signals was measured, as a dependence of mouse immunoglobulin applied to the blotting membrane. Labelled antibody (anti-mouse immunoglobulin) was applied in the total amount of 6.7 ng for each experiment. The applied amount of antigen was in the range of 0.1–0.5 ng.

Figure 4 Intensity of the Au signals depending on the amount of mouse immunoglobulin applied on the blotting membrane displayed in the colour-coded intensity scale



The Figure 4 shows not only that the ability of antibody to bind antigen was maintained, but also that the Au signal is proportionate to the amount of antigen present on the membrane.

CONCLUSION

Immunochemical methods are commonly used to analyse biological samples in both diagnostic and advanced research. They are able to specifically recognize the analyte in a complex matrix, such as a blood serum, or a histological section using a properly labelled antibody.

In this work, the immunoassay method is based on the preparation of the antibody bio-conjugate with suitable metal-based nanoparticles. The labelled antibodies are visualized with LA-ICP-MS after the immunoassay.

Experimentally, it has been demonstrated that by conjugating to 10nm nanoparticles the antibody does not lose its ability to bind the antigen. The method appears to be suitable for qualitative and quantitative analysis of low concentrations of analyte.

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