Multiplex Express In Vitro Diagnostics based on Magnetic Nanoparticles

P.I. Nikitin[*], N.V. Guteneva[*,**], A.V. Lunin[**], V.A. Bragina[*], S.L. Znoyko[*], A.V. Orlov[*,**], B.G. Gorshkov[*] and M.P. Nikitin[**]

[*]General Physics Institute, Russian Academy of Sciences, Vavilov St 38, 119991 Moscow, Russia, nikitin@kapella.gpi.ru

[**]Moscow Institute of Physics and Technology (State University), Institutskii per. 9, 141700 Dolgoprudny, Moscow Region, Russia

ABSTRACT

In this research, four different approaches to multiplex express biosensing have been developed based on the combination of magnetic nanolabels with an original technique of their quantification. The developed electronic detection methods are based on the highly sensitive technique of magnetic particle quantification (MPQ) by non-linear magnetization. A new generation of multi-channel MPQ readers has been developed that offer the record limit of detection (LOD) of 0.4 ng of MP in 0.2 ml volume within an extremely wide 7-order linear dynamic range. For the assay development we use a synergistic combination of electronic quantitative volumetric registration of magnetic nanolables with optical label-free monitoring of the kinetics of each step of immunochemical reaction, which is used for selection of the best assay reagents and regimes.

Keywords: magnetic nanoparticles, biosensing, immunoassay, highly sensitive detection, non-linear magnetization

1 INTRODUCTION

Magnetic nanoparticles (MP) have provided many breakthrough solutions for life science. The immense potential of MP as labels in advanced immunoassays stems from the fact that they, unlike optical labels, can be easily detected inside 3D opaque porous biosensing structures or in colored mediums, manipulated by an external magnetic field, exhibit high stability and negligible background signal in biological samples, etc.

The combination of magnetic nanolabels with an original technique of their quantification have permitted development of novel multiplex methods of express biosensing. Here we demonstrate four different approaches to multiplex assay for rapid simultaneous on-site detection of several antigens in complex matrices. The developed assays are based on readout of three-dimensional structures with an original portable multi-channel magnetic reader. The reader offers extremly highly sensitive detection of MP with magnetic particle quantification technique (MPQ) [1-3]. Recently these technique was used for MPQ-cytometry development for rapid quantitative determination of the oncological status of cells, as well as for assessment of antigen expression on cell surfaces [4].

2 MATERIALS AND METHODS

The developed multiplex biosensing platforms are based on registration of superparamagnetic nanolabels with magnetic particle quantification technique. The MPQ employs a nonlinear magnetization of superparamagnetic particles subjected to a magnetic field at frequencies f_1 and f_2 with recording the particle response at a combinatorial frequency that is a linear combination of f_1 and f_2 or higher harmonics of one frequency. The method is insensitive to linear dia- and paramagnetic materials. Importantly, its limit of detection (LOD) of MP is on the level of γ-radioactive techniques demonstrated for MP based on ^{59}Fe isotope.

A new generation of multichannel MPQ readers has been developed that offer LOD of 0.4 ng or 60 attomoles of MP in 0.2 ml volume within an extremely wide 7-order linear dynamic range. In this study, the readers have been adapted for each of the reported multiplex biosensing designs.

For development of the highly sensitive and rapid immunoassays, we carried out preliminary selection of optimal immunoreagents and implement their kinetic characterization using a biosensor based on the spectral-correlation interferometry (SCI) [5].

3 RESULTS AND DISCUSSION

3.1 Multiplex magnetic immunoassay on 3D porous filters

Multiplex biosensing has been developed based on simultaneous assay on the surfaces of several 3D filters. The polymer 3D fiber filters with immobilized capture antibodies were put into the pipette tips, and the multiplex assay was performed in one run by dosing the reagents with an electronic pipette (Fig. 1A).

The 50-nm magnetic nanoparticles were selected to be used as labels, and the signal was read out from the entire volume of the nontransparent 3D fiber filters employed as solid phase for sandwich immunoassays. Each filter of

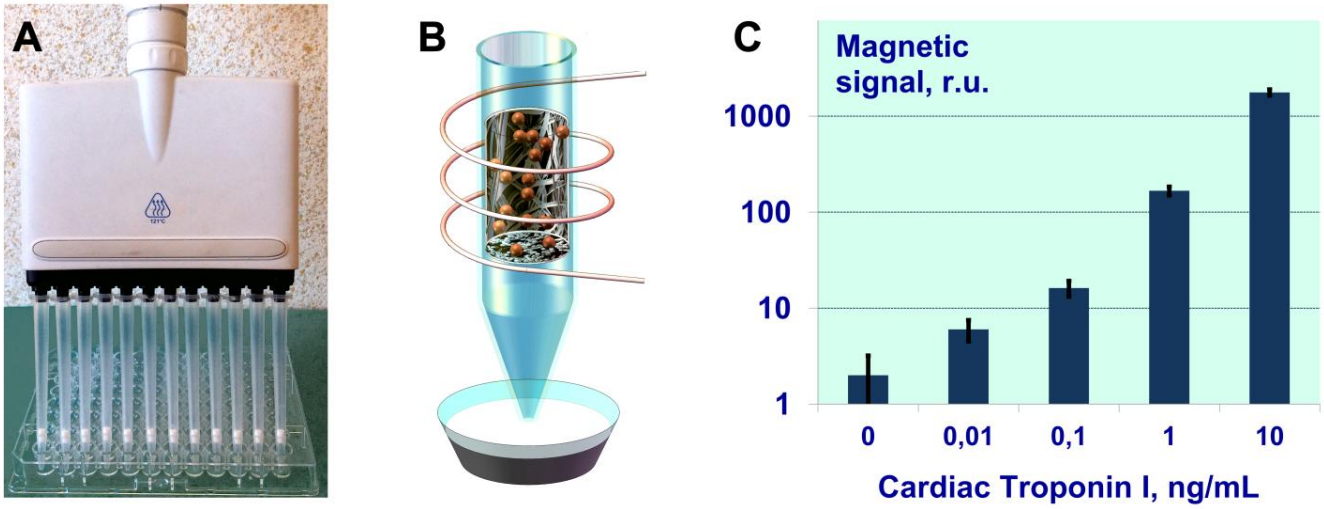

Fig. 1. Setup of multiplex magnetic assay on the surface of 3D filters: A – the filters are put into the pipette tips, the dosing of reagents is done with an electronic pipette; B – MP labels are MPQ-counted; C – dose-response dependence on concentration for cardiac troponin I.

small 30-µl volume provides large reaction surface of 20 cm^2, quick reagent mixing, as well as antigen immunofiltration directly in the course of the assay.

3.2 Multiplex sensing with magnetic biochips

A special MPQ reader has been developed for inductively interrogated magnetic biochips and counting of MP in several recognition spots on disposable biochips with various capture antibodies (Fig. 2). Such passive sensor chips can be attractive affordable consumables for medical diagnostics as compared to the single-used biochips that comprise build-in multilayer magnetic field sensors having contacts to readout results. With the developed inductively interrogated biomagnetic sensor chips, one can analyze small sample volumes < 10 µl.

The MPQ device intended for readout of planar chips or microfluidic cartridges comprises several small coils with radius R = 0.5 - 1 mm and a common single coil to generate magnetic fields at high f_2 and low f_1 frequencies, respectively. The experiments have demonstrated that the small coils can be successfully used to count MP inside a semisphere of the same radius. This fact allows separation of the main electronic units from the sensor chips or microfluidic cartridges, which can be interrogated from outside through 0.1 mm thick glass or plastic bottom.

The developed approach of inductive counting of MP is advantageous because it allows not only flat sensor chips traditionally used for MP counting by different build-in thin film magnetic sensors (GMR, GMI, etc.), but also those having more complex 3D-topology, for example, micropillar arrays, the height of which is comparable or less than the radius of small coils.

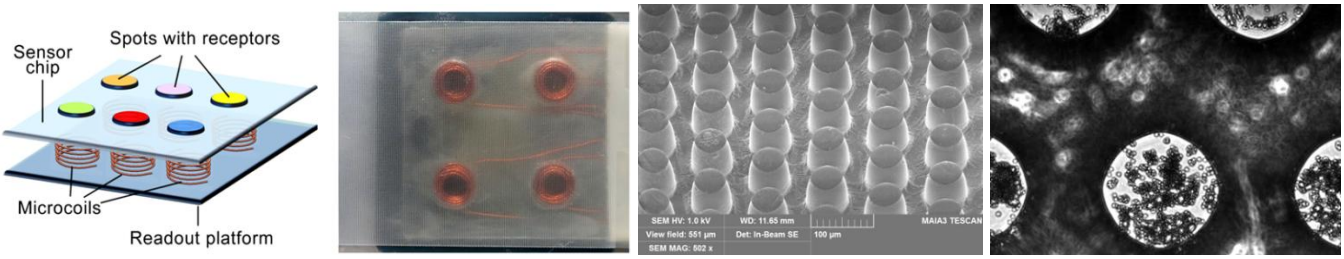

Fig. 2. Multiplex magnetic biochip (1); a photograph of disposable chip on top of the MPQ coils, which volumetrically count the MP in the chip spots (2); micropillar array substrate for chips (3) and a recognition spot with receptors after reactions with magnetic nanolabels (4).

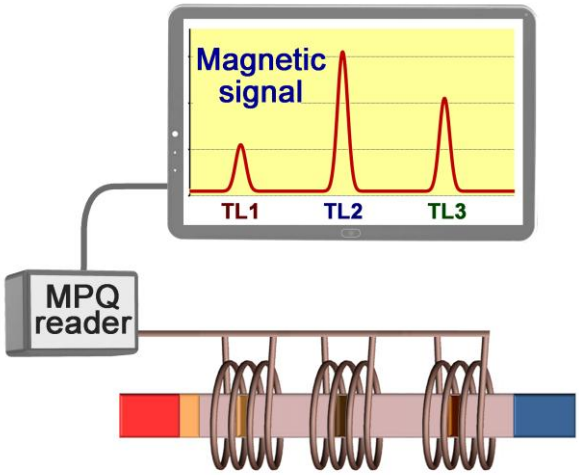

Fig. 3. Rapid multiplex magnetic assay based on quantitative lateral flow strips.

3.3 Multiplex biosensing by magnetic multi-line lateral flow strips

The approach was realized as a lateral flow test strip with several test lines. The MPs were simultaneously counted on each line independently by means of a multi-channel MPQ reader (Fig. 3).

It should be noted that such design of multiplex assay inevitably leads to the problem of cross-reactivity of immunoreagents because all the test components simultaneously present in the solution and can react with each other. Several different conjugates of magnetic particles interact with several antigens in the sample and several test lines on the strip.

Therefore, sensitivity and specificity of such test is traditionally several times worse than that of the respective single-plex assay. The approach discussed in the next section is completely free of this disadvantage. It can be realized solely due to employment of magnetic nanoparticles as the detectible labels in combination with the volumetric method of MP detection.

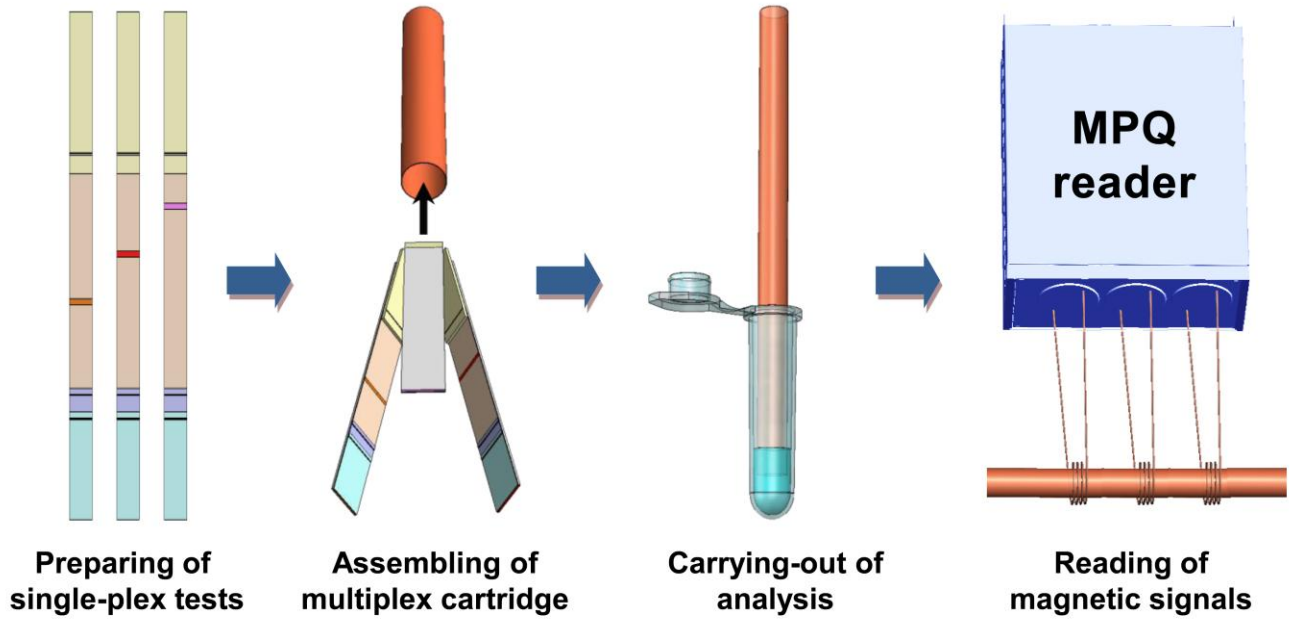

Fig. 4. Multiplex biosensing based on 3D modular architecture with several lateral flow tests of different specificity and detection of MP from all the tests simultaneously.

TechConnect Briefs 2018, TechConnect.org, ISBN 978-0-9988782-4-9

Besides, the proposed approach could be used for mapping of MP distribution. It is a tool for rapid, simple and cost-efficient optimization of all stages of the lateral flow assay without high consumption of reagents. The proposed method of recording quantitative distribution of magnetic nanoparticles along all constituent components of immunochromatographic lateral flow strips can be used for selection of proper concentrations of the used reagents such as amounts of Ab immobilized on MP, amounts of MP and conjugates deposited onto the test lines, etc.

The method could be also used for quantitative monitoring of total MP mass by determination of square under the curve of their distribution along the test strip. According to the experiments, this parameter did not depend on antigen concentration and remained constant for each batch of the test strips.

3.4 Quick on-demand design of multiplex magnetic biosensors based on 3D modular architecture

The setup of such multiplex test is shown in Fig. 4, that goes beyond the traditional planar techniques. The 3D design of several spatially separated tests of different specificity and simultaneous volumetric detection of MP from all recognition zones essentially simplify the requirements related to cross-reactivity of the reagents and functionalized MP, with virtually no sacrifice in performance compared with the single-plex tests.

Detection of cardiac and cancer markers, particular, of cardiac troponin I (cTnI) and prostate-specific antigen (PSA) as well as small molecules and oligonucleotides were used in the experiments. The LODs for detection of total PSA and cTnI in human serum was as good as 25 pg/ml and 12 pg/ml by dry-reagent magnetic lateral flow and express immunofiltration assays, respectively.

The analytical characteristics of the developed multiplex methods are on the level of the modern time-consuming laboratory techniques while assay time is less than 30 min. The developed multiplex biosensing platforms are promising for medical and veterinary diagnostics, food inspection, environmental and security monitoring, etc

4 CONCLUSIONS

Using highly sensitive magnetic nanoparticle quantification technique, we have demonstrated successful application to multiplex biosensing of 4 different approaches based on magnetic nanolabels: 1) one-run test on the surfaces of several 3D porous structures; 2) flat and micropillar microfluidic sensor chips; 3) multi-line lateral flow strips; 4) quick on-demand design based on modular architecture, which is the first 3D multiplexing method that goes beyond the traditional planar techniques.

The 3D design of several spatially separated tests of different specificity and simultaneous volumetric detection of MNP from all recognition zones essentially simplify the requirements related to cross-reactivity of the reagents and functionalized MNP, with virtually no sacrifice in performance compared with the single-plex tests.

The demonstrated detection of different types of analytes (cardio- and cancer markers, small molecules, oligonucleotides) confirms that the analytical characteristics of the developed multiplex methods are not inferior to those of the modern laboratory techniques. The developed multiplex biosensing platforms can be used for rapid, sensitive, simple and quantitative concentration measurements of various analytes in complex media and in wide dynamic ranges.

ACKNOWLEDGEMENTS

Different aspects of this multidisciplinary research were partially supported by the RFBR (grants No. 16-33-60228, 17-02-01415 and 17-54-560024); PRAS programs No. I.32 and I.40; and the Skoltech Systems Biology Fellowship (A.V.O.).

REFERENCES

[1]. M.P. Nikitin, V.O. Shipunova, S.M. Deyev, P.I. Nikitin, "Biocomputing based on particle disassembly," Nat. Nanotechnol. 9, 716-722, 2014.

[2]. A.V. Orlov, V.A. Bragina, M.P. Nikitin, P.I. Nikitin. "Rapid dry-reagent immunomagnetic biosensing platform based on volumetric detection of nanoparticles on 3D structures," Biosens. Bioelectron. 79, 423-429, 2016.

[3]. A.V. Orlov, S.L. Znoyko, V.R. Cherkasov, M.P. Nikitin, P.I. Nikitin, "Multiplex biosensing based on highly sensitive magnetic nanolabel quantification: rapid detection of botulinum neurotoxins A, B, and E in liquids," Anal. Chem. 88, 10419-10426, 2016.

[4]. V.O. Shipunova, M.P. Nikitin, P.I. Nikitin, S.M. Deyev, "MPQ-cytometry: a magnetism-based method for quantification of nanoparticle–cell interactions, " Nanoscale. 8, 12764-12772, 2016.

[5]. A.V. Orlov, M.P. Nikitin, V.A. Bragina, S.L. Znoyko, M.N. Zaikina, T.I. Ksenevich, B.G. Gorshkov, P.I. Nikitin, "A new real-time method for investigation of affinity properties and binding kinetics of magnetic nanoparticles," J. Magn. Magn. Mater. 380 (2015) 231–235.