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Nanomedicine: Nanotechnology, Biology, and Medicine
xx (2015) xxx–xxx
nanomedicine
Nanotechnology, Biology, and Medicine
nanomedjournal.com

Quantum dot-based lab-on-a-bead system for multiplexed detection of free and total prostate-specific antigens in clinical human serum samples

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Received 17 November 2014; accepted 3 March 2015

Abstract

An immunodiagnostic lab-on-a-bead suspension microarray based on microbeads encoded with quantum dots (QDs) has been developed and preclinically validated for multiplexed quantitative detection of prostate cancer markers in a representative set of human serum samples. The sensitivity and specificity of the microarray are similar to those of "gold-standard" single-analyte ELISA. Moreover, the array has an improved immunoassay capacity, ensures quantitative detection of multiple cancer biomarkers and may be operational in a considerably wider dynamic range of concentrations. The array is characterized by reduced time and cost of analysis and is compatible with classical flow cytometers. Proof-of-concept preclinical tests ensured simultaneous quantitative determination of free and total prostate-specific antigens in human serum, with clear discrimination between the control and clinical samples. The proposed approach to designing QD-based clinical microarrays is flexible and paves the way to development of a wide variety of immunodiagnostic assays for multiplexed early diagnosis of various diseases.

Key words: Fluorescent nanocrystals; Quantum dots; Suspension array; Multiplexed analysis; Cancer markers; Flow cytometry

Introduction

Cancer remains one of the leading causes of death in the world.[1] Early cancer biomarker screening and quantitative detection are crucial for improving targeted therapy. Screening tests for specific tumor serum markers have proved to be an effective method for detecting cancer in asymptomatic cases and are widely employed in clinical practice.[2]

Although individual tumor markers available today are not 100% specific and sensitive for disease progress,[3] the use of a panel of proven biomarkers (instead of single biomarkers) can significantly improve the sensitivity and efficiency of diagnosis.[4] Advanced multiplexed assays for simultaneous determination of different analytes in the same probe have recently become a useful and indispensable tool for cancer clinical diagnosis and imaging.[5-7]

At present, common approaches to multiplexed analysis of biological samples employ two-dimensional solid-state planar arrays[8,9] or liquid-state suspension arrays based on encoded microparticles.[10-13] Both detection systems have their specific advantages and shortcomings.[14-17] Solid-state immunoassays, such as ELISA provide relatively sensitive, specific, and precise quantification of tumor markers and are considered as "gold standards" of clinical practice. A serious limitation of ELISA is that it is essentially a single-analyte technology, where only one analyte can be measured in a single test. Although sequential

This study was supported through the Federal Targeted Program for Research and Development in Priority Areas of Development of the Russian Scientific and Technological Complex for 2014-2020 (contract no. 14.578.21.0054).

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http://dx.doi.org/10.1016/j.nano.2015.03.003
1549-9634/

ELISA tests for several analytes are feasible, they are expensive and time-consuming, require large sample volumes and complicated dilution procedures, and are operational in a narrow dynamic range of concentrations.[14,18] The solid-state planar microarrays can analyze thousands of proteins simultaneously, providing multiplexed profiling of markers,[9,19] but, unfortunately, the ranges of parameters measured and biomarker profile characteristics, sensitivity, and detection quality are limited by the planar matrix properties.[16,20,21]

Besides conventional solid-state planar microarrays novel technological approaches have recently been developed. Optical encoding principle allows realization of Förster resonance energy transfer (FRET) indicating highly specific analyte binding and significantly increasing specificity and sensitivity of analysis. For example, simultaneous time-resolved six-color FRET from one Tb complex (donor) to five different organic dye acceptors was performed for monitoring of multiple molecular binding events at very low concentrations, thus, providing quantitative detection of five cancer markers.[22]

The further development of multiplexed suspension arrays is currently of particular interest for clinical diagnostics. The advantages of liquid-state particle-based arrays over solid-state two-dimensional ones are determined by free motion of microparticles in three dimensions.[15] Therefore, suspension arrays are characterized by fast binding kinetics and high sensitivity and quality of analysis.[17,23] Moreover, they can be easily modified or combined to fit the target profiles.[10,14,24]

There are several available encoding schemes and, hence, principles of determination of a unique microparticle code in a suspension array.[25,26] The spectrometric encoding scheme is the most available and easy to use. This technology uses combinations of fluorophores incorporated into microbeads to obtain individual spectral codes. Such fluorophore-encoded beads can be rapidly analyzed by means of flow cytometry.[10,12,13,27,28]

Microparticles may be optically encoded with either classical organic dyes or semiconductor fluorescent nanocrystals ("quantum dots," QDs) of different colors (diameters). Although available organic dye-encoded beads are widely used for detection of a large panel of biomarkers,[11,13,23,24,29-31] specific optical characteristics of organic dyes limit the number of the possible unique color combinations in a detection array and often require complicated compensation schemes and complex equipment for multiplexed dye excitation and optical code read-out.

QDs have important unique advantages over classical organic fluorophores.[32] These are extremely high extinction coefficients accompanied by a significant photoluminescence quantum yield and, hence, a high brightness; narrow, symmetrical fluorescence peaks; the possibility to excite nanocrystals of different colors with a single light source; and an exceptionally high photostability.[33-36] Moreover, QDs can act as efficient donors for FRET to a suitable acceptor.[37] Among various types of QDs, core/shell CdSe/ZnS QDs have proved to be the most convenient tool for diagnostic and biomedical assays.[28,38,39] Semiconductor nanocrystals used as fluorescent tags improve the photostability, sensitivity and multiplexed imaging capacity of optically encoded beads. In addition, the use of QDs decreases the cost of antigen detection and simplifies both the procedures of multiplexed analysis using conventional flow cytometry, and data processing.[10,34]

Earlier, we reported on the first application of QD-encoded microbeads to proteomics, multiplexed antibody profiling, and clinical diagnosis of autoimmune diseases.[38] The proposed bead-based suspension array was designed for simultaneous identification of specific autoantibodies in the serum of systemic sclerosis patients. Besides evident advantages of optically encoded suspension immunoarrays the described system employs the FRET phenomenon in order to additionally improve the quality of analysis. The selective and highly specific detection is archived due to the energy transfer between microbead fluorescent tags (QDs) and visualization agent bound on the bead surface. Only in the presence of target markers the complete immune complexes are formed and energy transfer occurs to detect the amount of bound molecules.[38] This scheme allows to significantly decrease background signal and improve the sensitivity of analysis. The other QD bead-based suspension arrays have been recently designed and evaluated for the detection of virus and bacterial toxin markers in duplex and triplex format of analysis.[40,41] The described systems employ classical lab-on-a-bead principle of biomolecules immunodetection and provide simultaneous quantitative measurement of several biomarkers due to the unique optical and multiplexing properties of QDs.

Summarizing the current progress, the new generation of bead-based arrays encoded with QDs may enable efficient simultaneous determination of multiple antigens, enhance the clinical sensitivity and specificity of antigen screening in multiplexed diagnostics and become an advanced alternative to the conventional diagnostic approaches.

In this study, we have designed and tested a new multiplexed diagnostic system based on QD-encoded microbeads for simultaneous detection of prostate cancer markers in a representative set of clinical human serum samples. The detection of cancer serum markers is based on the formation of an immune complex of a specific capture monoclonal antibody (mAb), a target antigen, and a specific detector mAb on the surface of QD-encoded microbeads. The immune complex is visualized with a dye-tagged secondary detection agent, and the complete double-color lab-on-a-bead system is processed using conventional flow cytometry for simultaneous detection of free and total prostate-specific antigens (PSA). For the best of our knowledge, the system developed is the first example of a QD-encoded suspension diagnostic array enabling multiplexed quantitative detection of cancer markers in human serum and providing clear discrimination between the samples from cancer patients and healthy donors.

Methods

Optical encoding of microbeads with water-soluble QDs

Preparation of QD-encoded microbeads was performed using the adapted procedure of layer-by-layer deposition of alternatively charged polymer layers[42] and a layer of carboxyl-modified QDs.[43] CdSe/ZnS core/shell QDs coated with trioctylphosphine oxide (TOPO) were synthesized, solubilized and modified with derivatives of polyethylene glycol (PEG) (Thermo Fisher Scientific, USA) containing both thiol and carboxyl groups using adapted protocols.[44,45] (see Supplementary Materials).