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Nanomaterials for early detection of cancer biomarker with special emphasis on gold nanoparticles in immunoassays/sensors

R. Viswambari Devi [a,b], Mukesh Doble [b,*], Rama S. Verma [a,*]

[a] Stem Cell and Molecular Biology Laboratory, Department of Biotechnology, Indian Institute of Technology, Chennai 600036, India
[b] Bioengineering and Drug Design Laboratory, Department of Biotechnology, Indian Institute of Technology, Chennai 600036, India

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ABSTRACT

At the onset of cancer a selective protein or gene based biomarker gets elevated or modified in body fluids or tissues. Early diagnosis of these markers can greatly improve the survival rate or facilitate effective treatment with different modalities. Though the sophisticated imaging technologies like Magnetic Resonance Imaging, Positron Emission Tomography and Computed Tomography have the impact of nanotechnology on their improved performance, they are however unsuitable for early detection of cancer biomarkers or their quantification. Other approaches for cancer diagnosis based on cell morphology and microscopy (biopsies) are too not conclusive for early diagnosis of cancer. The only hope for early diagnosis of cancer in near future is by the detection of cancer biomarkers using immunoassays/sensors that are reformed by Nanotechnology. Attractive properties of nanoparticles have miraculously lifted up the design, fabrication, sensitivity and multiplexing of these immunoassays/sensors in biomarker detection. With this aspect we have explored the recent advancements in immunosensing techniques that were developed exploiting the unique properties of gold nanoparticles. We have also discussed the possible future trends with respect to gold nanoparticle-coupled microfluidic sensors; paper based analytical devices and the single-molecule biosensing.

Contents

1. Introduction

Nanotechnology is the fast expanding area of research focussing on the development of novel diagnostic and treatment strategies in cancer therapy. It promotes the development of devices

* Corresponding authors.
E-mail addresses: mukeshd@iitm.ac.in (M. Doble), vermars@iitm.ac.in (R.S. Verma).

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for detection, diagnosis, drug delivery and ablation therapies for cancer. Inorder to provide effective cancer treatment, early detection of disease is crucial. So the efficacy of cancer treatment depends on the detection of the biomarker at an early stage as well as with accuracy (Kierny et al., 2012). Biomarkers are biological molecules (including proteins, peptides, nucleic acids) found in the tissues, blood and other body fluids (Yang et al., 2014). They get altered during abnormal processes including cancer and hence can be monitored to differentiate affected patient from a normal person (Nahavandi et al., 2014). Genomics and proteomics have generated a variety of biomarkers but however only few of these stand out as superior diagnostic tools, and even fewer get validated and approved (Polanski and Anderson, 2006). Comprehensive reviews are available on different cancer biomarkers (Bhatt et al., 2010) and herein we highlight only detection of cancer antigens based biomarkers. Prostate specific antigen (prostate cancer), alpha-fetoprotein (hepatocellular carcinomas), carcinoembryonic antigen (colorectal cancer), cancer antigen 125 (ovarian cancers) are some important cancer antigens that serve as diagnostic and prognostic biomarkers of their respective cancers. Detection of these biomarkers is challenging as they occur in ultralow levels and hence require ultrasensitive detection methods such as immunoassays or immunosensors. These methods are the most sensitive diagnostic techniques for the quantitative detection of tumor markers due to their highly specific molecular level interaction between an antigen and antibody.

An immunoassay is a biochemical test and an immunosensor is an analytical device, both used for detecting and measuring the concentration of an analyte (like a cancer antigen biomarker) in a given sample (Cruz et al., 2002). Most of the immunoassays/sensors designed for cancer biomarker detection take the advantage of sandwich type antigen–antibody reaction, where the analyte of interest is between the immobilised primary capture antibody and the secondary detection antibody with or without labels (Pei et al., 2013). Different types of labels exist that allow detection through different means. Labels can be enzymes (alkaline phosphatase and horse radish peroxidase that catalyzes, the hydrolysis of phosphate groups from a substrate molecule and the oxidation of a substrate respectively resulting in colored product or emits light (chemiluminescence)) or fluorophores (fluorescein emits fluorescence). On immunocomplex formation, the labels conjugated to detection antibodies yield a characteristic signal that is detected by a suitable spectrophotometric technique. The intensity of the label signal is related to the amount of analyte in a sample of given volume. In an immunosensor, the recognition element is coupled to a transducer to convert a biological interaction into measurable electrical signal that can be easily processed, recorded and displayed. Certain immunoassays/sensors developed have the advantage of detecting the analyte interaction in real-time (label free), by transducing a change in one or more physical parameters (refractive index, mass change) into electrical signal, thus avoiding the indirect approach of using labels.

Success of immunoassays/sensors for ultrasensitive detection of cancer antigen biomarkers depends on the reaction efficiency between antigen and antibody, immobilization of antibodies (recognition element) on the support surface, the choice of transducer and signal probes (Andreotti et al., 2003; Madersbacher and Berger, 2000; Yu et al., 2015). Antibodies with higher affinity immobilized on support with large surface-to-volume ratio can have greater specificity and sensitivity with minimum cross reactivity (Yu et al., 2015). Immobilization of recognition element, transduction efficiency and signal amplification are greatly improved and enhanced with nanomaterials. Various nanomaterials like noble metal nanoparticles, quantum dots, carbon nanotubes, graphene and various nanocomposites are being successfully employed for ultra sensitive immunoassays/sensors.

The use of nanomaterials in immunoassays and sensors provides (i) high surface area for the attachment of antibodies and thus facilitating better access of analytes to these antibodies, (ii) signal amplification, and (iii) label-free-real-time protein detection. Hence, using nanomaterial in fabrication of sensors and assays will lead to ultrasensitive detection of ultralow levels of cancer biomarkers. Research efforts and success towards achieving excellent detection methods for cancer antigen biomarkers, focusing on prostate specific antigen (PSA) and alpha fetoprotein (AFP) using nanomaterials (highlighting gold nanoparticles) are reviewed here.

2. Gold nanoparticles (AuNPs) in cancer antigen biomarker detection

In nanotechnology, miniaturization of bulk materials to nanomaterials (at 1–100 nm scale) provide increased relative surface area, quantum confinement and hence unique properties. Among various nanomaterials, noble metal nanoparticles like AuNPs are the most compatible for use in clinical diagnostics (Howes et al., 2014). They have been widely investigated for their potential applications in biomarker detection because of their remarkable plasmonic properties and large surface to volume ratio (Eustis and El-Sayed, 2006). The optical properties of AuNPs are related to the phenomenon called localized surface plasmon resonance (LSPR). LSPR originates from the resonance collective oscillation of valence electrons in the conduction band of metallic nanoparticles, on interaction with electromagnetic field. This excitation of electrons results in subsequent selective photon absorption and surface enhanced optical phenomena including Raman scattering, fluorescence and various non-linear effects (Tauran et al., 2013). LSPR in AuNPs also possess high refractive index (RI) sensitivity, close to the metal surface and hence serves as an attractive biosensing platform for label-free-real-time protein detection.

LSPR, high surface area, controllable morphology, high stability, biological compatibility, easy functionalization are the interesting features of AuNPs. These properties are highly dependent on their size, physical dimensions, spacing and their surrounding environment. Thus AuNPs form an excellent transduction platform for biosensing. They are the most employed nanoprobes (Xu et al., 2014; Tiwari et al., 2011) in immunoassays/sensors fabricated based on Raman scattering, Resonance Rayleigh scattering, fluorescence and chemiluminescence (Fig. 1).

3. LSPR based immunoassays

Optical immunoassays are usually designed with biological recognition interface (target analyte specific primary antibody) on the surface of a nanomaterial such as AuNP. Binding of the target analyte (biomarker) will cause changes in the plasmon absorbance that will be transduced into an optical signal which is measured by a suitable optical technique.

When light incidents on a metallic nanosurface, the electron oscillation (LSPR) scatters the light either at the same frequency (elastic scattering-Resonance Rayleigh scattering) or at a shifted frequency (inelastic scattering-surface enhanced Raman scattering) with increase in intensity (Israelsen et al., 2015). The intensity of the scattered light is measured by suitable optical techniques (eg. Raman spectroscopy, Rayleigh scattering microspectroscopy).

3.1. Surface enhanced Raman scattering (SERS)

Detection of cancer biomarkers at ultralow level was impossible by conventional Raman spectroscopy, since the intensity