Nanotechnology in proteomics: Current status, promises and challenges

Mehrnaz Mostafavi [1]*, Jalaledin Ghanavi[2]

[1] Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] National Research Institute of Tuberculosis & Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

*Corresponding author: email address: mz_mostafavi@yahoo.com (M.Mostafavi)

ABSTRACT

In genomics, the ability to amplify rare transcripts has enabled rapid advances in the understanding of gene expression patterns in human disease. The inability to increase the copy number and to detect the signal of rare proteins as unique species in biological samples has hindered the ability of proteomics to dissect human disease with the same complexity as genomic analyses. Advances in nanotechnology have begun to allow researchers to identify low-abundance proteins in samples through techniques that rely upon both nanoparticles and nanoscale devices. This review describes some of the physical and chemical principles underlying nanomaterials and devices and outlines how they can be used in proteomics; developments which are establishing nanoproteomics as a new field. Nanoproteomics will provide the platform for the discovery of next generation biomarkers. The most promising candidates for nanoproteomics, namely carbon nanotubes and nanowires, quantum dots and nanoscopic gold particles, offer several advantages such as high sensitivity, real-time measurements and improved reproducibility.

Keywords: Nanobiotechnology; Nanomaterials; Nanoparticles; Nanoproteomics; Biomarker

INTRODUCTION

1.1. Proteomics

Proteomics has witnessed rapid growth over the last decade, with increasing emphasis on development of robust and high-throughput technologies to understand the diverse proteome. Researchers have gone beyond traditional techniques and approached promising disciplines like nanotechnology to satisfy the growing demands of studying numerous complex and functional proteins. Applications of nanotechniques in proteomics have steadily been growing over the years and it has established itself as a technical platform for sensitive detection of low abundance proteins in shorter time. The main focus of this inter-disciplinary approach is to increase the sensitivity and improve biocompatibility. The most promising candidates for nanoproteomics, namely carbon nanotubes and nanowires, quantum dots and nanoscopic gold particles, offer several advantages such as high sensitivity, real-time measurements and improved reproducibility.Proteomics, the study of proteins expressed by a genome, is now an established field. Since the term was first coined at a scientific conference in Italy by Wilkins in 1994, proteomics has gone through successive revolutions of method optimization and technology development. The key has always been in the development of high throughput methods. With the introduction of immobilized pH gradients 2-DE has become the workhorse of protein separation and a quick method for obtaining protein expression patterns. Up to 10 000 distinct protein and peptide spots can be separated on one gel [1, 2].

1.2. Nanomaterials

Inorganic nanomaterials, whose features are controlled at the nanometer scale (1 to 100 nm) possess many unique and advantageous physical properties when applied as ultra-sensitive signal transducers and protein concentrators in the fields of molecular diagnostics and proteomics. When the dimensions of materials are reduced to the nanometer regime, their intrinsic physical properties can be determined by their size and shape as well as composition, in ways often not predictable from either their component atoms or from the properties of micrometer sized particles of the same composition [3]. This will be

exemplified and explained in the following sections with specific consideration being given to superparamagnetic nanoparticles and their primary use in the magnetic separation of bio-molecules [4], quantum dot (QD) (semiconductor) and plasmonic (metallic)nanoparticles as improved labels and optical reporters [5-7] and nanowires (NWs) as label-free, real-time biosensors [8].

1.3. Nanoproteomics

Nanomaterials have long played a part in proteomics, however the difference between these earlier nanomaterials and the new nanomaterials is the level of understanding of the relationship between their structure and the properties of their materials leading to the ability to engineer the latter to suit the applications much better.In the near term nanotechnology will have a major impact on proteomics and diagnostics (as well as imaging and therapy). In molecular diagnostics the impact will lead to new devices and sensing modes whilst in proteomics it will extend and augment existing methods.

As a result, it is still impossible to detect all protein molecules existing in a biological material. This is the main problem of proteomics. The second problem of proteomics is the high dynamic range of concentrations of protein molecules existing in biomaterial, the so-called dynamic concentration barrier.So proteomics needs new technologies that make it possible to register single molecules in the presence of high abundant molecules. Nanotechnology can play a role in these new technologies. Even now, these technologies have the ability to register and visualize single macromolecules and their complexes, as well as single nanoparticles. Among the nano-technologies are methods of scanning electron microscopy, near field scanning microscopy and other microcanteliver techniques, and nanowire and nanopore detectors. [9,10]

The second approach that will enable the detection limit as well as the dynamic concentration barrier to be overcome are technologies that, like the polymerase chain reaction, can multiply single protein molecules. Such technologies are currently being developed, and without any doubt, their success will depend on the predominance of nanotechnologies in this sphere because manipulating single molecules is the subject matter of nanotechnology.The development of methods such as nanoelectrophoresis and nanochromatography gives hope that these technologies will deal with single molecules and not with concentration parameters; in other words, nanopreparative technology concerns the separation of single molecules, their detection and visualization. At the same time, of course, it is important to recognize that the current technologies of proteomics, such as multidimensional electrophoresis, multidimensional chromatography in combination with mass spectrometry in its modern types, as well as methods of quantitative estimation of proteins in biomaterials,are so highly developed that they should be retained unchanged and new methods using new technology should simply be introduced. At the same time,existing nanotechnologies on the way to converting protein microarrays into nanoarrays can undergo significant development.

With the solution of this problem, proteomics will attain a new technology that seems to be more efficient than technologies already in existence.Summarizing the reasons for introducing a nanotechnology section into *PROTEOMICS*, there are two of the main problems of proteomics:the first is fundamental and lies in the detection of the number of proteins molecules in a biomaterial, in analogy with genomics, and the second is applied and lies in the detection of protein biomarkers for different diseases.

The first problem will be solved after the creation of high-throughput detectors for counting single molecules instead concentration detectors.The second problem seems to be polysemantic, namely biomarkers for diseases of the cardiovascular system, in particular cardiac infarction, do not need nanotechnologies [9]

2. Nanoproteomics and biomarker discovery

Biomarker discovery requires sensitivity and a comprehensive coverage of bio-molecules which may represent the alteration caused by the disease of interest. Protein candidate biomarker discovery often involves identifying proteins found at extremely low concentrations. As highlighted in

the previous sections there are three main areas where tradi-tional proteomics is unable to offer adequate solutions for biomarker discovery and diagnostics. Firstly in the separation of complex protein mixtures, second, the detection and identification of low abundant components and third, the ability to analyze samples with a dynamic range above 3.5 orders of magnitude. there are only two main areas where nanoproteomics has been used in biomarker discovery: nanostructured surfaces for the enhancement of proteomic analysis *via* MS and RP protein microarrays; and nanoporous materials for selective binding and fractionation of proteins and protein fragments.

2.1. Nanostructured surfaces

MALDI-MS has been the most widely used method for protein identification using suitable organic matrixes. Although these matrices have been widely used there are several issues which need improvement. The most obvious problem is the interference of matrix peaks in the low molecular weight region which means that peptide data cannot be collected below 800 *m/z*. The other problem is finding the suitable matrix for the sample. To solve these problems, the use of several kinds of metal and metal oxide particle has been reported in the literature, such as Al, Mn, Mo, Si, Sn, TiO2,W, WO3, Zn, and ZnO [10], sol-gel-deposited TiO2 [11],ordered mesoporous WO3-TiO2 [12], Au-NPs [13], and selfassembling Ge nanodots [14]. Apart from metal and metal oxide nanoparticles, carbon nanotubes have also been used [15].

Nanotechnology techniques used in semiconductor processing has been applied to create MALDI-MS targets for matrix free ionization such as column/void-network silicon thin films prepared by plasma-enhanced chemical vapor deposition [16], single-crystal silicon NWs deposited on silicon wafer [17], ordered arrayed silicon nanocavity that was lithographically fabricated on a silicon wafer [18] and ordered arrays of submicrometer groove structures [19].

Another example of using nanostructured surfaces, is a study by Gaspari *et al*. [20] in which silica-based nanoporous surfaces were used to capture low molecular weight peptides from human plasma. The surface was fabricated by coating silicon chips with a 500 nm thick nanoporous film of silicon oxide. The average pore size was estimated to be about 7 nm.Harvested peptides were analyzed by MALDI-TOF MS and 70 peaks were found in the 800–10 000 *m/z* range. This method was able to detect peptides in the ng/mL concentration level. Tailoring of the surface allows selective enrichment of specific protein or peptide classes. This study used the matrix-free desorption/ionization on silicon method first reported by Wei *et al.* [21].RP protein microarrays are also using nanostructured surface for proteomics. These arrays enable the highthroughput screening of PTMs of signalling proteins with diseased cells. In a recent report by Geho *et al*. [22] used QDs as reporter agents for the amplification of microarray sensitivity taking advantage of their multiplexing potential.

Instead of nitrocellulose, which has high intrinsic autofluorescence,silicon was used as a potential microarray surface. Through semiconductor etching techniques, large surface areas can be created on silicon to enhance protein binding.

2.2. Nanoporous materials

In search for a reproducible and highly specific method for the isolation of representative protein signatures of a particular disease state, researchers looked for technological advancement in fractionation and reproducible binding of characteristic molecules from body fluids. One of the new solutions which emerged as a result of this is the use of nanoporous material surfaces as a fractionation tool for serum-based analysis and biomarker discovery [23,24,25,26]. Geho *et al*. for example used nanoporous silicon wafer to selectively deplete a fraction of proteins from serum. By controlling the pore size of nanoporous glass beads, distinct subsets of the proteome were harvested. The protein and peptide profiles of fractionated and unfractionated serum samples were compared by SELDI-MS.

Nanoporous materials are also used in chromatography using monolith supports. For an overview of monoliths in proteomics technology see a review from Josic [27]. The combination of high flow rates, high surface area and well established surface modification procedures makes monolithic columns and capillary packings

suitable for many aspects of proteomics from sample preparation to enzymatic digestion to peptide separations. One of the strengths of monoliths is the ability to tailor the pore sizes and distributions to match the particular application. Rainer *et al*. [27,28] used monolithic capillary columns for fractionation of broad range serum proteins and peptides which were separated using an immobilized metal ion affinity chromatography column. The eluted peptides were identified by MALDI-TOF MS.

3. Nanotechnology and molecular diagnostics

As medical diagnostics is more and more relying on molecular markers and highly specific therapies targeted at disease specific receptors, novel methods are emerging for the detection and quantification of low abundant bio-molecules.
Diagnosis requires selectivity and quantification. "Selectivity"refers to how well an assay can detect particular molecules in a complex mixture without interference from other molecules. Biomarker validation and measurement on the other hand depends on accurate and reproducible protein quantitation.The emergence of novel nanotechnologies not only increased sensitivity as it was highlighted in the previous section but also enabled biologists to achieve high specificity by enriching very low abundant proteins from complex mixtures.This section gives examples of nanomaterials applied in the field of biomarker measurement and clinical diagnostics enabling cheaper and more accurate solutions for Point-of-Care (PoC) home-tests.Building on the acquired knowledge, nanomaterials are now ready to be employed in the field of molecular diagnostics (Table1), taking it from hospital laboratories to the patient [29].

3.1. Au-NPs

Au-NPs are considered as the industry-standard in nanotechnology.First introduced to the PoC diagnostics market in the form of a pregnancy test and now adopted by a range of PoC lateral-flow immunochromogenic tests, such as Phadia's ImmunoCAP Rapid test for specific IgE against ten common allergens, and Merck's Singlepath and Duopath tests for food borne pathogens, Au-NPs still serve as one of the few examples of a successful integration between the area of nanotechnology and PoC diagnostics. Au-NPs are chosen above dyed latex beads as their higher diffusion rate results in improved mixing of the analyte and capture particle,and an increase in sensitivity. Their small size and large molar absorption coefficient also leads to the formation of a dense line of highly visible particles forming at the capture line, providing clearer read-out. [29]

Table1. Applications of nanotechnology to medical diagnostics, adapted from Leary *et.l.* [37]

Nanotechnology derivative	Function	Features
Au-NPs	Detection of DNA and proteins	Rapid test with simple and clearer optical read-out
SERS	Detection of proteins, small molecules and DNA	Label free detection or SERS active tags
QDs	Detection of proteins, FRETs and immuno-histochemistry labelling	Increase in sensitivity over conventional assays, multiplexed immunoassays
Magnetic immunoassays	Detection of DNA and protein	Highly sensitive sandwich detection method with a high dynamic range
Bio-barcode assay	Detection of DNA and protein	Au-NPs and magnetic microparticles bind targets with extremely high sensitivity
NWs	Detection of DNA, proteins and cells	NWs are functionalized with appropriate antibodies
Nanocantilevers	Detection of genes, RNA, proteins, bacteria, viruses	Highly sensitive mass sensors, can be functionalized with biologic molecules

3.2. SERS

Gold (as well as silver) nanoparticles have also played a major part in SERS, another optical technique with widespread applications in molecular diagnostics. Two SERS formats are commonly used as signal transducers in diagnostics; labelfree SERS assays where the SERS spectrum of the analyte itself is measured [30]**,** and the use of SERS reporter tags in sandwich immunoassay formats [31,32].

An example of the latter, are Nanoplex-Biotags, which consist of Au-NPs coated with unique SERS-active reporter molecules that are then protected and made biocompatible with a silica shell.As well as label-free formats based on antibody coated SERS active substrates [30] the *in vivo* quantitative measurement of glucose has also been achieved.

3.3. QDs

Rather than offering a new paradigm in molecular diagnostics, QDs offer important improvements in established fluorescent-based diagnostics and sensing. Due to the advantageous spectral properties of QDs *versus* organic fluorophores,QDs improve the multiplexing capabilities, detection limits and robustness of fluorescence methods .With the development of thin-film GMR and Spin Dependent Tunnelling materials–materials whose resistance is modulated by external magnetic fields–extremely sensitive,miniaturized magnetic sensors have been fabricated [33,34].

For molecular diagnostic applications, these have often been in the format of magnetic immunoassays that detect analytes bound between magnetic nanoparticles and a magnetic sensor. An applied external field is used to induce a magnetic moment in the superparamagnetic nanoparticles,creating a local magnetic field that is detected by the sensor.

3.4. NWsensors

NWsensors operate on the basis that the change in chemical potential accompanying a target/analyte binding event, such as DNA hybridization or protein binding can act as a fieldeffect gate upon the NW, thereby changing its conductance .In an elegant proof-ofprinciple study it was demonstrated that boron-doped silicon nanowires (SiNW) could be used as highly sensitive, realtime,electrically based sensors for biological and chemical species [35]. To this end, Zheng *et al*. [8] described a multiplexed electrical detection of cancer markers using SiNW field-effect devices in which distinct NWs and surface receptors are incorporated into arrays (see Fig.1).

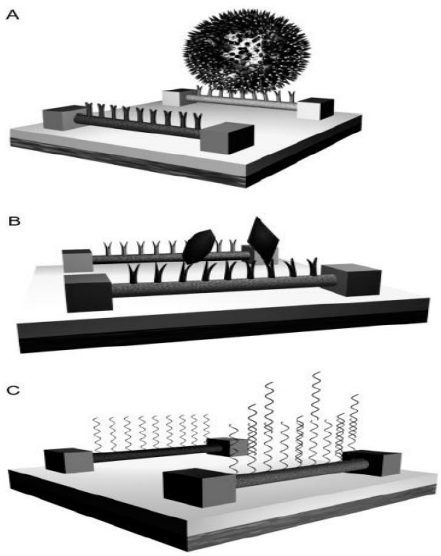

Figure1. NW-based detection of single molecules. (A) Schematic showing of a NWdevice with antibodies on the surface capturing a single cell; (B) same as A capturing proteins and (C), NW device capturing DNA molecules. Adapted from [8]

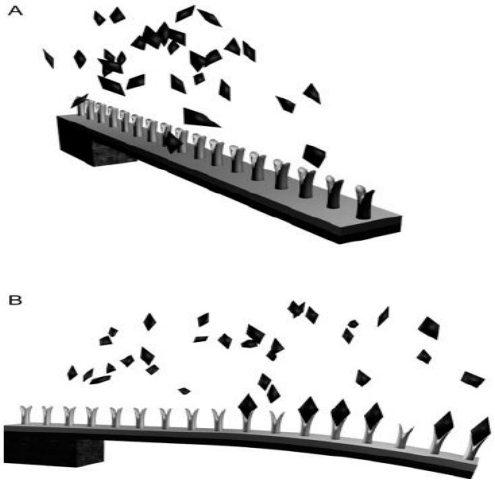

Figure 2. Functionalized manocantiliver array. (A) Schematic illustration of a silicon nanocantiliver array with receptor molecules on the surface and target proteins above; (B) same as before after protein binding showing the cantilever band owing to the applied force of the binding event. Adapted from [38].

3.5. Nanocantilevers

Nanocantilevers are the most simplified nanoelectromechanical systems based device and are an example of how low-cost silicon micro-fabrication technology can produce nanostructures whose size-dependant mechanical properties form the basis of a label-free biosensing platform with multiplexing capability. As described earlier, analyte binding to nanocantilevers results in a measurable deflection due to changes in surface stress (as illustrated schematically in Fig.2). Such devices have recently been used for the detection of cardiac biomarkers [36].

CONCLUSIONS AND OUTLOOK

Proteomics provides methods for correlating the vast amount of genomics information that is becoming available with the equally vast protein information that is being produced through analysis of cells under normal *versus* altered states.The development of nanotechnology, for example in the area of nanoparticles demonstrates that research is rising to this challenge, as these technologies will revolutionize the direction of proteomics research in future.Looking at where nanotechnology is currently and where it may be in the future in relation to proteomics and diagnostics we can distinguish two trends; evolution and revolution.The evolutionary trend uses nanotechnology to improve current approaches by providing materials and devices with improved performance but used in a conventional fashion.

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