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APPLICATIONS OF NANOPROTEOMICS IN BIOLOGICAL SYSTEMS: A REVIEW

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ABSTRACT

The high-throughput techniques used in nanoproteomics generally outperform in comparison to normal proteomics techniques. Approaches like disease biomarker detection in humans are one of the major achievements, which have led to the detection of different biomarkers for autoimmune, infectious, neurodegenerative and cardiovascular disease. The use of novel sensors and different nanoproteomic approaches also helps in identifying biomarkers for different types of cancer and other diseases. In plant biology, nanoproteomic assists in gene transfer, creation of deoxyribonucleic acid crystals and identification and quantification of total protein. It also has application in microbial proteomics research. Using nanoproteomics has also helped in studying allied scientific areas like proteobionics and secretomics. This review article covers the current application and future prospects of nanoproteomics approaches.

KEYWORDS: Biological Systems, Disease Biomarker, Nanoparticles, Nanoproteomics

INTRODUCTION

Nanoproteomics is the amalgamation of nanotechnology and proteomics. Methods related to nanotechnology and proteomics play a major role in research and development but deciphering certain processes at the nano level has always met with difficulties when considering any single broad aspect between nanotechnology and proteomics. The main problem that still exists with proteomics is that it is not possible to detect all the protein molecules present inside a biomaterial. Another issue with proteomics is the dynamic concentration barrier, which is about the high dynamic range of concentration of protein molecules that exists in a biological material. These are some of the reasons due to which proteomics needs new technologies that will facilitate it to register single molecules in the presence of highly abundant molecules. Nanotechnology plays a significant role in these new technologies as it cans the ability to register and visualize single molecules and their complexes, as well as single nanoparticles. The existing nanotechnological methods are scanning electron microscopy, near field scanning microscopy, microcantilever techniques, nanowire and nanopore detectors [1, 2].

NANOPROTEOMIC APPROACHES IN DIFFERENT FIELDS

2.1 Disease Biomarker Detection: Advancement in proteomics has led into the discovery of disease biomarker by coupling high-throughput techniques with novel nanosensors. Reports on biomarkers have also focused on their detection limit and their application for the detection of autoimmune diseases, infectious diseases, neurodegenerative diseases and cardiovascular diseases. Some of the various biomarkers used in the diagnosis of different diseases using nanoproteomics are HSA (Diabetes) [3], Proteinase-3 (Wegener Granulomatosis) [4], HBV Virus (Hepatitis) [5], Anthrax protective antigen (Anthrax) [6], ADDL (Alzheimer) [7], Tau protein (Alzheimer) [8], CRP, TNFα and IL-6 (Inflammation, CVR) [9]. Application of nanoproteomics has also become a part of cancer research. Different

nanoproteomics approaches have been applied for biomarker detection in cancer diagnosis. AFP (serum) [10], CEA (serum) [11], PSA (serum) [12], CA15-3 (blood) [13], PSA-ACT Complex (serum) [14], EGFR (cancer cell lines) [15] and SK-BR-3 (breast cancer cell lines) [16] are some of the biomarkers detected using gold nanoparticles (Au-NPs). Different studies using quantum dots (QDs) for cancer diagnosis have also revealed biomarkers as Mucin1 (epithelial cancer cells) [17], CD44v6+ and CD24- (breast cancer cells) [18], PSCA and HER2 (cancer cell line) [19], Nucleolin & Integrin αvβ3 (cell line) [20], PSA (purified protein) [21] and few more. Carbon nanotubes (CNTs), used for cancer diagnosis has also found biomarkers like AFP (serum) [22], Volatile organic compounds biomarker (breath) [23], PSA, PSMA, platelet factor & IL-6 (serum) [24] and few more. Silicon nanowires are yet another approach for biomarker detection in cancer research. VEGF (blood) [25], PSA, PSAα1-antichymotrypsin, CEA and mucin-1 (serum) [26] and CRP (serum) [27] are some of the biomarkers detected through silicon nanowires. Other major techniques used for biomarker detection are nanomechanical resonators [28], suspended microchannel resonators [29], optofluidic ring resonator sensors [30], microcantilevers [31], silicon photonic microring resonators [32], 2D cantilever array chip [33] and silver nanoparticles.[34, 35]. Physical and chemical principles about nanomaterials and their devices and how they can use in proteomics has already been reported earlier [36]. Application of nanotechnology in molecular diagnostics has increased as medical diagnostics rely on molecular markers and highly specific therapies targeted at disease specific receptors [36].

2.2 Nanotechnology Derivatives: The nanotechnology derivatives having different applications in biological system e.g. in biomedical diagnostics, in agri- biotechnology, bio-sensor technology etc. *(i) Au-NPs* (gold nanoparticles):important nano-derivatives, as researchers have developed an integrated proteomics approach using chemically functionalized gold nanoparticles as a novel probe for affinity purification to analyze a large protein complex *in vivo*. This approach has been applied to globally map the transcriptional activation complex of estrogen response element (ERE) and have been designated as quantitative nanoproteomics for protein complexes (QNanoPX) [37]. Multifunctional gold nanoparticles can be used for diseases diagnosis and therapy [38] by forming complexes as cyclic peptide-capped gold nanoparticles (CP-AuNPs) [39] which acts as prodrugs for delivery of anticancer and antiviral agents. pH Low Insertion Peptide (pHLIP) [40] is yet another technology, which targets gold nanoparticles into tumors and is beneficial for radiation oncology and imaging. Gold Nanoparticles also acts as potential antimicrobial agents for *Escherichia coli* and *Salmonella typhi* [41]. (ii) *SERS* (Surface-enhanced Raman scattering spectroscopy) has proven to be highly efficient as it provides a lot of information about the chemical structure of probed substances.

It has its wide application in medical diagnosis such as detection of human breast cancer cells [42], potential noninvasive nasopharyngeal cancer [43] and studies of frozen and deparaffinized tissue section of pediatric tumors [44-47]. (iii) *QDs* (Quantum dots) are generally nanoparticles of a semiconductor material. Usually they are sulfides or selenides of metals like zinc or cadmium, eg. ZnS or CdSe. Quantum dots are continuously getting adopted by life science and biomedical communities as it has a lot of applications in biology and biomedicine. Various applications of quantum dots in the medical field are tumor targeted bioimaging [48], delivery of vitamin D to tumors in case of breast cancer, detection of Parklinsons disease at early stage [49], QDs-based fluorescent biosensing for proteins and nucleic acid [50] as well as its conjugation with carbon nanotubes has been used for diagnostic purposes [51-52]. (iv) *Magnetic Immunoassay*: Magnetic beads have replaced the conventional, enzymes, radioisotopes and fluorescent moieties in case of magnetic immunoassay. It is a novel type of diagnostic immunoassay, involving the specific binding to an antibody for its antigen, and a magnetic label is conjugated to one element of the pair.

The application of magnetic immunoassay in food-safety control has also proved successful as it helps in detection of a bacterial toxins [53]. Quantitative analysis of intracellular proteins produced within cells is also possible using paramagnetic particles [54- 55].

It has been also reported the field of remanence generated by magnetic markers utilized during magnetic immunoassay can be measured with superconducting quantum interference device (SQUID) [56]. Detection and quantification of cell-surface antigen using magnetic nanoparticles is yet another possibility, which helps in early diagnosis of diseases like cancer [57]. Medical diagnostics, food pathogen detection and testing water sample are the three major application of magnetic immunoassay [58]. (v) *Bio-barcode assay*: Scientific reports have also mentioned about a bio-barcode assay which is a highly sensitive technique for detecting target proteins and nucleic acids. Detection of specific targets is also possible with use of Dithiothreitol (DTT) induced ligand exchange [59]. Gold nanoparticles are generally used for this assay, but it can also be performed using polystyrene (PS) microparticles which are used in case of fluorophore-labeled barcodes [60].

A protein of interest can also be detected at attomolar level using a colorimetric bioassay [61]. Based on sensitivity of this assay prostate-specific antigen (PSA) was earlier detected at a concentration as low as 500 aM [62] but a report of 2009 has also mentioned about a bio-barcode PSA assay which is approximately 300 times more sensitive than the commercially available immunoassays [63]. Latest reports have also mentioned the application of this assay in detection of neurotransmitter such as dopamine [64]. Further on the basis of detection of nucleic acid's targets this assay has also been used for simultaneous detection of four types of virus DNA using capillary DNA analyzer [65], for detecting two targets DNAs using a label-free bio-barcode assay [66] and for detecting DNA of microbes such as *Salmonella enterica* which is a major cause of foodborne illness [67]. Latest reports have mentioned about its sensitivity for dual-aptamer recognition [68] and development of an aptamer-based bio-barcode assay for screening anti-cancer drugs [69]. (vi) *NWs* **(**Nanowires) often called nanowhiskers or nanorods are very minute, solid and crystalline rods measuring between 10-100 nm in diameter and around few micrometers in length. Preparation of nanowire and its application in detecting target molecules is in trend for quite a couple of years. Molecular imprinted polymers (MIPs) using alumina nanoporous membranes have shown a good binding ability between the imprinted site and template protein molecule.

The reason behind the binding ability is that the imprinted sites are either close to the surface or at the surface of MIPs due to which it can recognize the sites of the target protein [70]. A review on nanowire sensors has also mentioned about its importance in medical and life sciences. These sensors have unique capabilities in binding to a target protein which can act as a major breakthrough in the discovery and development of potential pharmaceutical thus acting as a tool for drug discovery.

These sensors also have key properties of detecting DNA and monitoring its enzymatic elongation. Apart from these, they can also be used as devices for detection of multiple disease marker proteins and single viruses [71, 72]. Delivery of cytotoxic agents into target cells is also possible using silica nanowires (SiNWs). Using silica nanowires Shiga toxin type 1A subunit (StxA1) was inserted into cultured bovine and human epithelial cells, and this technology can be used as an immunotherapy for different diseases [73]. Silica nanowires have also been configured as field-effect transistors (FETs) and are extremely sensitive for electrical detection of proteins [74, 75]. Approaches using vertically aligned nanowires fixed to the surface for biosensing, electrophysiology and drug delivery has also been successful [76]. Microbial nanowires has also been reported in *Geobacter sulfurreducens* where the nanowires are none other than its own pili, which

helps in transferring the electrons that are formed after obtaining energy for its growth by oxidizing organic compounds [77]. A latest report has also mentioned about formation of functional nanowires from amyloid peptides as it has a natural tendency to self-assemble into nanofibrillar structures. Researchers have also shown that Ni-NTA coated nanowires have better protein purification efficiency and are more stable in the form of dry powder [78]. (vii) *Nanocantilevers* – Nanocantilevers are rectangular shaped thin strips of silicon of few nanometers in width used as sensors for detecting and capturing bio-molecules. Earlier researchers used to work with microcantilevers and bioassays have been carried for prostate-specific antigen (PSA) [79]. Using this technology, as a result of surface stress produced from antigen-antibody reaction the biomarker proteins can also be detected [80]. It can also be used for converting the biophysical and biochemical processes into signals that can be recorded [81]. In context to the improvements required in nanoscience, nanocantilever has come forward as a bioanalytical device that is portable, label free and be operated at a rapid pace. Thus, it helps in detection of proteins, nucleic acids, viruses and bacteria [82, 83]. This technology has emerged as a low-cost and ultra-sensitive alternative technique in comparison to the existing optically detected and chemical techniques. Cantilever arrays have also been used in detecting mutations in BRAF gene [84]. It is a gene in humans that produces a protein called B-Raf. Latest reports have also mentioned about development of a cantilever based protein biosensor that can detect antibodies [85].

2.3 Biomarker Detection Using Sensors: Latest reports on nanoproteomics approaches have discussed throught the study of label-based [86] and label-free detection systems [87], indicating their advantages and applications in biomarker discovery. Under label-based detection, protein microarrays has been employed as an alternative to conventional ELSA procedures due to its high-throughput characteristics. Protein microarrays studies for prostate cancer have detected five potential protein biomarkers-vonWillebrand factor, IgM, α1-antic-hymotrypsin, villin and IgG. Cyanine dyes (Cy3 and Cy5) are among the most common fluorochromes employed for protein microarray detection due to their brightness and reduced complexity of labeling proteins with charged lysine residues. Bead-based arrays can also be used for the kinetic characterization as well as the interaction of enzymes with multiple substrates in a multiplexed analysis. Stable isotope labeling with amino acids in cell culture (SILAC) is a label technique which is analyzed by mass spectrometry [88]. In spite of the wide use of label-based techniques researchers are focusing on label-free techniques, as they are cleaner, faster and simpler. Most commonly used label-free techniques in proteomics are - relative quantification by the peak intensity of LC-MS, relative quantification by spectral count and absolute label free quantification. Biomarkers related to metabolic diseases have also been included for diseases like diabetes mellitus, gout, hyperuricaemia, Lesch-Nyhan syndrome and chronic liver disease. Although there is immense progress in the field of biomarker discovery, but it is still getting encountered with technological and biological challenges like difficulty in detection of low-abundance protein, dynamic range of protein concentration and extreme variations between individuals [89].

2.4 Nanoproteomics and Plant Biology: Nanotechnology has also proved valuable in the plant biotechnology sector. Production of transgenic plants is always considered superior as compared to that of normal cultivar. Moreover, the transgenic technology is considered as an important tool in plant research and has an extensive application in agricultural research and formulation of phytomedicines. Earlier strategies included *Agrobacterium* transformation and also by some physical techniques like microprojectile, electroporation, etc. Latest nanotechnology includes direct gene transfer with the help of nanoparticles. Currently, carbon nanotubes (CNT), liposomes and positively charged liposomes (lipofectin) are used as novel technologies for gene transfer [90]. Carbon nanotubes with the immobilized cellulose act as an efficient DNA delivery system in plant cells [91]. CNTs on the other hand, can also penetrate the seed coat and dramatically affect

germination and growth of plants. Treatment of soil with nanoparticles like zinc oxide and cerium oxide has revealed that these nanoparticles get accumulated on the soil and can reduce the productivity of plants. Researchers have also found a way of converting plant matter into building blocks of common plastics using a nanotechnology process that offers an alternative to oil-based production.

In comparison to existing plastic bags which are produced from crops such as corn and sugar and have a very limited use, the new system produces chemicals similar to petrochemical works and can be used in a wide range of industries. Application of nanotechnology in crop biotechnology includes the successful creation of DNA crystals by producing synthetic DNA sequences that can self assemble into a series of a three dimensional triangle like pattern. This structure can help in crop improvement by organizing and linking biomolecules like carbohydrates, lipids, proteins and nucleic acids to these crystals. It has also been observed that for isolation of mannan /mannose binding proteins from dilute samples, and its concentration by nano-liquid chromatography (nano-LC) and capillary eletrochromatography (CEC) is also possible with use of monolithic capillary columns with mannan immobilized in it [92]. A group of researchers have also inserted carbon-coated iron nanoparticles in pumkin plant and it was used as nanodevices for plant pathological treatment [93]. Based on their uses a microarray of microRNAs (miRNAs) collected from roots and leaves of rice seedlings has also been analyzed [94]. Quantitative and qualitative characterization of lupin proteins from four cultivars of *Lupinus albus* has also been carried using microfluidic nano HPLC-Chip coupled with Ion Trap mass spectrometer and has proven to be a very sensitive technique [95].

A recent report has also mentioned about carboxylate-terminated carbosilane dendrimers, which are used as nanoadditives for separating different proteins present in olive and soyabean seeds [96]. Specifically in relation to food-related allergies, pulp proteins of Avocado (*Persea americana)* has also been studied using nanoscale liquid chromatography coupled to tandem mass spectrometry (nano-LC-MS/MS) through combinatorial peptide ligand libraries (CPLL) [97]. Nano-LC-MS/MS technique has also been used to study the proteins associated with changes in *Oryza sativa* when the plant gets infected with Rice Yellow Mottle Virus (RYMV) [98], for assessing the different kinds of proteins present in the xylem, and phloem saps [99] as well as for studying the different kinds of proteins present on the leaves and roots of rice plant [100]. Nano UPLC-MS^E is yet another technique, which has been used for identifying and quantifying the total protein present in soyabean seeds [101].

2.5 Other Important Approaches: Current proteomic approaches have been divided into two categories – mass spectrometry and Non-mass spectrometry based. MALDI-TOF (Matrix-assisted laser desorption/ionisation-time of flight), atmospheric pressure-MALDI, LC/MS/MS (Liquid chromatography–mass spectrometry), 2D gel/MS (two-dimensional gel - mass spectrometry) and nanomate are some of the mass spectrometry based techniques whereas 2D gel electrophoresis, multi-dimensional protein identification technology (MudPIT), protein arrays, two-hybrid systems and isotope-coded affinity tagging are non-mass spectrometry based techniques. All these techniques have their own merits and demerits [102- 107]. Nucleic Acid Programmable Protein Arrays (NAPPA) are yet another approach, which utilizes a complex mammalian cell-free expression system to produce proteins *in situ.* This method is an alternative to fluorescent-labeled approaches. It appears to have the capability of analyzing protein function and protein-protein interaction in studies promising for personalized medicine [108]. NAPPA is capable of probing with unique sensitivity native *in situ* protein-protein interactions and also helps in identification of key proteins involved in the control of cancer and its proliferation [109- 110]. Atomic force microscopy (AFM) has been introduced as a powerful characterization

platform which provides valuable insights about the surface proteome of microbial cells. In current microbial proteomics research, analyzing microbial cell-surface proteins is an exigent task which has a major application for drug design, vaccine development and microbial monitoring [111-112].

ALLIED SCIENTIFIC AREAS

Research and development associated with nanoproteomics has also led to the emergence of new areas/branches in this field. Few researchers have also mentioned about proteobionics which they have considered as a new branch of nanoproteomics. In proteobionics, biomimetics have been introduced into proteomics and it is about the study of primordial proteins synthesized by nanobacteria [113]. Secretomics is yet another area where proteins secreted by cell, tissue or organism are getting studied with the support of proteomic and nanoproteomic approaches. An in-depth study of the secretome of hepatocellular carcinoma cells has been done where the protein enrichment is performed using nanometer-sized Linder's type L Zeolite crystal (nano-LTL-zeolite), protein fractionation through 1-D SDS-PAGE and finally protein purification though LC-ESI-MS/MS [114]. Researchers have also observed that when endothelial cells are exposed to cigarette smoke, they generally respond by secreting some secretome which when analyzed using nano liquid chromatography coupled with high-resolution mass spectrometry revealed about the unique peptides present in it [115].

Using nanoproteomic approaches, it has also come into light that nanomaterials have been very successful in the detecting biomarkers and has a lot of applications in food and beverages sector [116]. Latest reports on application of nanomaterials include cancer nanomedicine [117, 118], usefulness of melatonin nanoparticles [119], synthesis and ability of silver-doped titanium TiO_2 powder-coated surfaces [120], surface morphology of $ZnAl_2O_4$ ceramic materials [121], electrospun nanofibers for drug delivery applications [122], antimicrobial activity of Hippurate nanocomposite [123], PEI-siRNA nanocomplex for liver cancer therapy [124], properties of C-15 nanopeptide and its effect on HeLa cell line [125], effect of aluminium silicate nanotube coating on bone regeneration [126], application of gold nanorod [127, 128], action of gold-coated magnetite nanoparticles in murine breast cancer [129], magnetic nanoparticles in regenerative medicine [130] as well as for screening of C-reactive protein for diagnosing cardiovascular disease [131], interaction of nanomaterials with biological systems [132], activity of silver nanoparticles on bone surgery devices [133], importance of nanomagnetically labeled immunoassay in clinical diagnosis [134], antibody-cojugated Rubpy dye-doped silica nanoparticles for detection of *Vibrio cholera* O1 [135], even nanoparticles entered into a human body through nanomedicines often helps in medicial imaging [136], detection of pathogenic viruses using synthesized silica-coated magnetic nanoparticles [137], antifungal effect of hydroxyapatite decorated with silver (HA@Ag) nanoparticles [138], use of nano-hydroxyapatite/collagen/poly (L-lactic acid) (nHAC/PLA) composites as a vascularized bone substitute for grafting [139], antitumor activity of Chitosan nanoparticles (CS-NPs) [140], vascular tissue engineering through Chitosan/collagen scaffold [141], ectopic bone formation with help of injectible Chitosan/Nanohydroxyapatite/Collagen composites [142], silica nanaoparticles in drug-delivery systems [143] and correlation of silver nanoparticles and chitin powder for antimicrobial activity [144]. Personalized medicine is yet another developing sector where with the help of nanomaterials even stem cell tracking has become possible [145].

CONCLUSIONS

With respect to traditional techniques related with proteomics, nanoproteomics have lots of technical advantage. Diagnosis of human diseases has become a whole lot easier with application of nanotechnology and has become a

trendsetter. Nanoproteomics has been so much potential that it is even possible to detect a solitary molecule with a protein. Use of nanoparticles like manganese oxide, perfluorocarbon and combination of QDs with magnetic iron oxide also helps in medical imaging like MRI. Medical devices can even be miniaturized like a heart pacemaker. But safety concerns still exist with the usage of nanoparticles as far as *in vivo* use of nanoparticles is concerned due to toxicity. Nanoproteomic's approaches have also proven beneficial with its successful utilization in microbial proteomic studies and also in relation to plants. This approach will definitely have a broad range of application in near future and will overcome most of the drawbacks for the existing technologies.

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