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Application of Proteomic Tools in Modern Nanotechnological Approaches Towards Effective Management of Neurodegenerative Disorders

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Abstract: Neurodegeneration is the progressive loss of structure or function of neurons leading to neuronal death, usually associated with ageing. Some of the common neurodegenerative disorders include Alzheimer's disease, Parkinson's disease, Creutzfeldt-Jakob disease, and Huntington's disease. Due to recent advancements in high-throughput technologies in various disciplines such as genomics, epigenomics, metabolomics and proteomics, there has been a great demand for detection of specific macromolecules such as hormones, drug residues, miRNA, DNA, antibodies, peptides, proteins, pathogens and xenobiotics at nano-level concentrations for in-depth understanding of disease mechanisms as well as for the development of new therapeutic strategies. The present review focuses on the management of age-related neurodegenerative disorders using proteomics and nanotechnological approaches. In addition, this review also highlights the metabolism and disposition of nano-drugs and nano-enabled drug delivery in neurodegenerative disorders.

Keywords: Disposition, nano-applications, nano-drugs, nano-techniques, nanotechnology, neurological disorders, proteomics.

1. INTRODUCTION

The term neurodegeneration is generated by a combination of two different words, *'neuro'* meaning *'nerve cells'* and *'degeneration'* meaning *'progressive damage'*; and can be applied to various conditions that result in continual loss of neuronal structure and function finally leading to neuronal death [1]. Common neurodegenerative disorders (NDDs) include Alzheimer's disease (AD), Parkinson's disease (PD), Creutzfeldt-Jakob disease, frontotemporal dementia and Huntington's disease [2]. Progressive accumulation and aggregation of proteins like tau, α-synuclein and amyloid-β (Aβ) have been reported to be involved in the gradual development of various NDDs [3, 4]. Unfortunately, to our knowledge, there is no single drug available that can halt or even slow down the progress of brain degeneration caused by NDDs. Apart from adverse effects on human health, NDDs have been reported to exhibit significant associations with other chronic diseases like cancer, diabetes and cardiovascular diseases [4-8]. Many different approaches have been proposed and utilized to develop cure of these chronic diseases [9-13], but full proof treatment options are still obscure. This calls for an urgent need to develop accurate and informative diagnostic tests as well as effective therapeutics for the devastating health burdens which could be based on new technological advancements such as proteomics and nanotechnology.

Proteomics deals with the identification, quantification and characterization of the total protein content present at a given time to help understand life at molecular level by development of novel therapeutic agents and diagnostic tools to provide insights for new biotechnological advancements [14]. The skills, experimental approaches and technological platforms supporting proteomics research are rapidly evolving [15]. Therefore, the application of novel qualitative and quantitative nanotechnological findings in the study of NDDs at different levels of neuronal circuit have the potential to help elucidate the biochemical pathogenesis of neurodegeneration and aid in the discovery of new biomarkers [16, 17].

The field of nanotechnology is an amalgamation of chemistry, engineering, biology and medicine [18]. According to the National Cancer Institute (NCI), nanotechnology includes the utilization of various technologies such as nanoarrays, protein arrays, nanopore technology, nanosensors and immunoassays involving nanoparticles (NPs) which have great potential to transform modern medicine in terms of diagnosis and treatment of diseases [19]. Analyses at nanolevel concentrations promise better efficiency, rapidity, low running cost and small sample volume requirement, all of which are indispensible in the latest platforms of proteomics, glycomics and metabolomics [20]. Therefore, the future potential lies in proteomics-based discovery and detection of nano-enabled biomarkers. A rational combination of proteomics with nanotechnology offers greater hope for therapeutic advances that could ameliorate NDDs [21, 22].

Recent advancements in high-throughput genomics and proteomics technologies can aid scientists to develop nano-analytical techniques capable of detecting hormones, drug residues, RNA, DNA, antibodies, peptides, proteins, pathogens and xenobiotics at nanolevel concentrations [23]. New methods and techniques such as protein conjugation with wheat germ agglutinin, cationic moieties (cationization), antibodies and nanogels are being developed for targeted-delivery of drugs and biomacromolecules to the central nervous system (CNS) for treatment of NDDs [24, 25]. Since the use of a number of approaches in understanding the pathogenesis and management of NDDs has not yielded promising results so far, this review focuses on the potential usage of modern proteomics

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and nanotechnological approaches towards a better understanding and management of NDDs. A brief outline of the metabolism and disposition of nano-drugs and nano-enabled drug delivery system is also presented.

2. PROTEOMICS ASPECT OF NDDs

NDDs are pathologically characterized by the progressive formation of lesions composed of disease-specific misfolded proteins [4, 26]. Antemortem prediction of NDDs' pathology is often challenging due to overlapping features of their clinical syndromes [27]. AD is an age-related NDD which is histopathologically-characterized by the presence of neurofibrillary tangles (NFTs), senile plaques (SP) and loss of synapse [25]. Over the years, AD has been reported to be one of the most prominent NDD affecting almost 28 million people worldwide [28]. AD is a progressive brain disorder characterized by an irreversible loss of neurons and diminished intellectual abilities such as memory and reasoning thus hampering social or occupational functioning of affected individuals [1]. This chronic illness progresses rather slowly for many years and can manifest in a variety of neurological and psychiatric disorders [29, 30]. The amyloid cascade hypothesis has dominated the field of AD for many years with modern approaches to counter the disease often ending in failures [31]. Over the years, many other hypotheses have also been proposed for the pathogenesis of AD, but none can solely account for overall dimension of this deforming disease [32, 33]. Hence, the exact mechanism of AD pathogenesis still remains elusive.

AD is characterized by certain molecular signals which can only be successfully diagnosed during post-mortem thus further impeding the early diagnosis [34]. Amyloid plaques and NFTs have been implicated as possible neurodegenerative agents of AD [35]. In addition, AD patients are also characterized by a decrease in the brain volume [36]. The main proteinaceous constituent of amyloid plaques is Aβ, which is 40–42 residue long peptide derived from amyloid precursor protein (APP) [37]. It is believed that APP is deposited at nerve terminals with some studies indicating its role in axonal trafficking [38, 39].

PD is another very prominent and debilitating NDD, which is also characterized by the presence of neuronal loss [40-44]. In PD, degeneration of the midbrain nigrostriatal dopaminergic neurons occurs, thus affecting several important motor symptoms leading to rigidity, bradykinesia, hypokinesia and a resting tremor [45]. Like AD, the exact cause of PD is also not well established. Many factors such as reactive oxygen species (ROS) formation, neuroinflammation and protein misfolding have been implicated in the development of PD [46]. For example, the presence of Lewy bodies, sporadicity and loss of dopaminergic neurons in the substantia nigra are the major characteristics of PD [47]. Owing to the significant research work conducted in the past few decades, the underlying mechanisms of PD are attributed to the right identification of a number of gene-encoding proteins such as α-synuclein, parkin, ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), PARK6, PTEN-induced kinase 1 (PINK1) and DJ-1 [48-53]. These genes have been implicated in protein misfolding, development of oxidative stress and impairment of the ubiquitin-proteasome system [48-55]. In addition, inherited genetic mutations and the corresponding proteins α-synuclein [56], DJ-1 [57], parkin and *PINK1* [58] have also been reported to be involved in the pathology of PD.

Many NDDs, especially the PD, are characterized by the presence of α-synuclein clumps in nerve cells. In some familial forms of PD, mutation in the α-synuclein gene [56, 59] and accumulation of α-synuclein protein in the brain have also been reported [60]. For example, a German family with PD was reported to exhibit a missense mutation, Ile93Met, in the *UCHL1* gene [61]. This mutation slightly affects the catalytic activity of the thiol protease and may disturb the normal proteolytic pathway and protein aggregation [62-64] thus suggesting that abnormal aggregation of α-synuclein protein may also be a potential causative factor for PD. Mutations in another protein (*PINK1*) have been reported to be responsible for hereditary early-onset PD [58]. Two homozygous mutations in the putative serine/threonine kinase domain of the PINK1 gene were identified by sequence analysis of candidate genes among PD patients [58]. It was postulated that these mutations may affect substrate recognition or kinase activity thus laying the foundation for the onset of PD [58].

In addition to the above, the pathogenetic mechanisms of many NDDs have been attributed to altered phosphorylation [65, 66]. For example, reports of α-synuclein phosphorylation (serine 129) in the Lewy bodies in human brains with synucleinopathy further suggests the potential role of altered phosphorylation in the pathogenesis of PD [67]. It is postulated that PINK1 may phosphorylate mitochondrial proteins in response to cellular stress in order to confer protection against mitochondrial dysfunction [52, 58, 68]. It has also been reported that, PINK1 is upregulated in cancer cells due to the presence of tumor suppressor gene PTEN [69]. In the neurons, PTEN signaling pathway is reported to promote excitotoxin-induced apoptosis in the hippocampus by regulating cell cycle and cell migration [70]. However, PINK1 was not reported to show any significant effects on PTEN-dependent cell phenotypes [69] thus calling for further investigation on its actual contributory role in the PTEN pathway. The proteins involved in the pathogenesis of AD and PD are summarized in (Fig. **1**).

3. PROTEOMIC TOOLS IN NANOTECHNOLOGY

Over the years, many proteomic tools have been modified according to their usage in nanotechnological methods with the most prevalent being the nano-chromatography and nano-electrophoresis (Fig. **2**).

3.1. Nano-Chromatography

Nano-chromatography is a combination of chromatographic and capillary electrophoretic separation methods showing high sensitivity (detection of at least up to ng/L). Two types of nano-chromatographic methods are discussed below.

3.1.1. Nano Liquid Chromatography (NLC)

The concept of NLC was introduced by Karlsson and Novotny in 1988. NLC is defined as the chromatographic modality involving samples in nanolitres with detection at the level of ng/ml [71]. NLC is usually performed on a chip and is also known as "lab-on-chip" chromatography. Tubular or packed capillaries with mass spectrometer detector are used to obtain separations in nano-level concentrations. The internal diameter size of the NLC columns are usually 50-100 μm and offer a good separation potential, hence, they are strategically coupled to micro- and nano-electrospray mass spectrometry [72-74].

Siviero *et al* presented an innovative, reliable and completely automated approach for generation of NLC gradient where the system is electronically-controlled with multi-position valve hosting six loops, each filled with several different mobile phase compositions [75]. It has a low flow rate and valve actuation facility, reducing solvent consumption by 40 times, thus making it attractive since it is economical and environment-friendly as well.

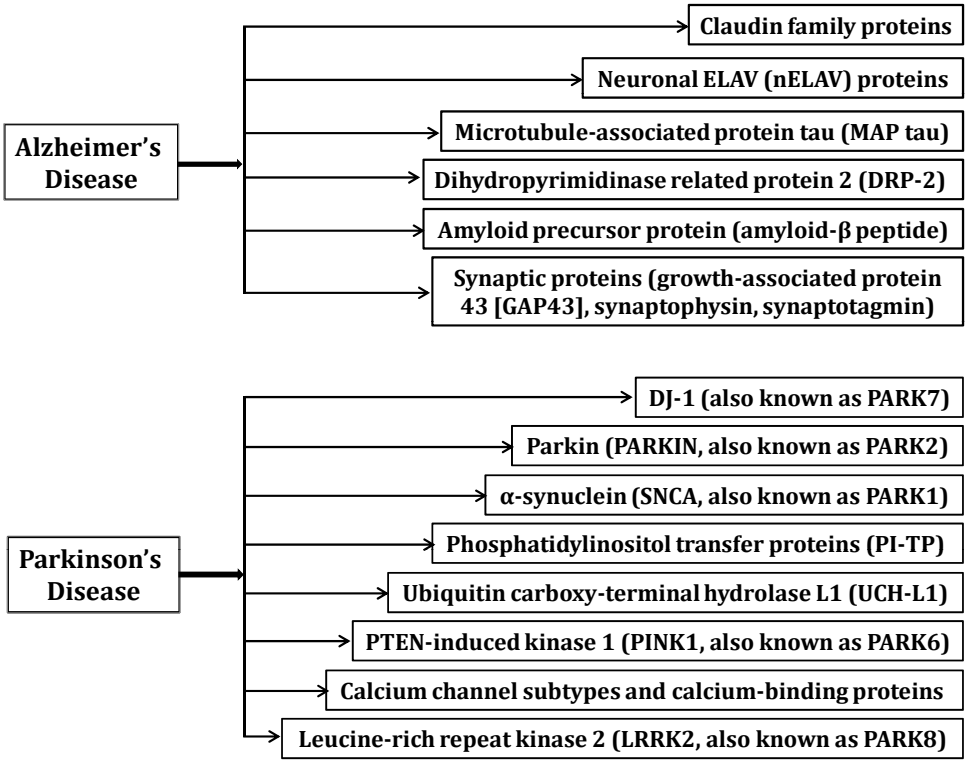

Fig. (1). Proteins involved in the pathogenesis of AD and PD.

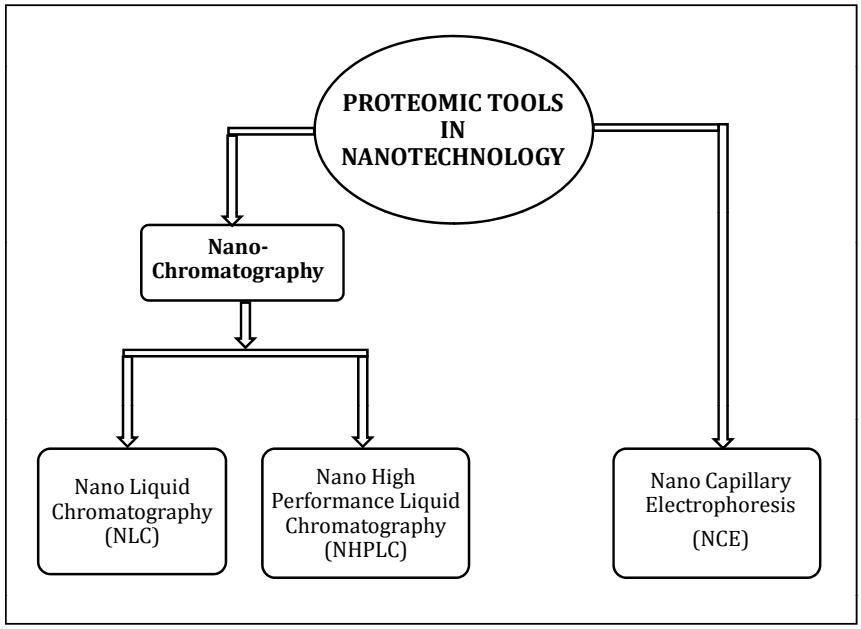

Fig. (2). Various proteomic tools that can be applied in nanotechnology.

3.1.2. Nano High Performance Liquid Chromatography (NHPLC)

A chip-based analytical system which is capable of bearing pressures of between 13 and 150 bars with pressure- or voltage-driven nanochromatography has been developed by Szekely and Freitag allowing microflow sensing and calibration compensation, thus ensuring consistent performance [76]. The mobile phase reservoirs applied in NHPLC are small, air-tight, contaminant free containers made of high quality glass. The mobile phase flows in a constant laminar fashion and usually consists of water/acetone or methanol mixtures. The reproducibility and accuracy of its flow rate are precisely controlled by a microchip flow sensor which is characterized by a high precision, digital intelligence and excellent reliability (Dionex Corporation, USA).

NHPLC employs high grade polyether ether ketone tubings having diameters in micrometer range with minimum connection gaps that can provide a constant laminar flow and minimal void volume. The flow rate of NHPLC pumps ranges between 25 to 4,000

nL/min. A microchip-based nano-flow sensor has also been designed using a nano-flow splitting technique to generate a slow flow (Dionex Corporation, USA) [77]. In another design, the pressure of compressed air and the flow sensor have been employed for precise nL/min flow rates thereby ensuring an excellent feedback control system (Eksigent, California, USA) [78]. Alternatively, a low dispersion injection method and micro-autosampler can further yield sharp peaks with improved chromatographic resolutions. Overall, the selection of column inner diameter and length and chip is dependent on its applications. Nevertheless, on-chip pressure-driven NHPLC technology still requires further refinement to combine various parts of NHPLC system, especially the separation column, continuous monolithic bed and NP-coated column [79-81] for better efficiencies.

In another study, HPLC coupled to electrochemical detection and nano electro-spray ionization double quadrupole orthogonal acceleration time of flight mass spectrometry were used for identification and quantification of plasma levels of endogenous morphine and anti-inflammatory cytokine, i.e., interleukin-10 as well as adrenocorticotropin levels in PD patients [82]. The study demonstrated that enhanced motor skills and mood elevation is seen with cyclical exercises leading to alleviation of some of the clinical characteristics of PD.

3.2. Nano Capillary Electrophoresis (NCE)

Proteomic analysis especially of low abundant proteins is tedious and exigent. Fortunately, the recent advent of innovative microfluidic devices such as NCE can help ease the challenge [83, 84]. Protein digestion by enzymatic treatment plays an important role in the sample pre-treatment process. Usually, proteins are fragmented into small peptides prior to analysis. The chip-based capillary electrophoresis (CE) involve fused silica capillaries thus allowing nano-level analyses of low quantity samples or samples present in minimal concentrations for genomic, proteomic and drug development studies. It comprises a microchannel network for pre- and post-sample handling, reactions, separation and identification. NCE is characterized by sample injection and electrolyte flowing at nano-level rate thereby reducing sample volume and imparting high speed and improved separation efficiencies [85, 86].

A new portable microchip electrophoresis, equipped with a high voltage power supply having dual amperometric (DC or pulsed) detection capability, a bipotentiostat and a chip holder has been designed for *in situ* analysis using microchips signal transduction by electrochemical detection [87]. Its performance was reported to be better than other commercial gadgets for separation of neurotransmitters, epinephrine, 3,4-dihydroxy-L-phenyl-alanine and dopamine.

A two chip-based NCE system was microfabricated by Phillips *et al* for analyses of inflammatory neuropeptides in body fluids [88]. Dual chips are designed to perform electrokinetic flow immunoassays by utilizing an immunoaffinity port containing an array of immobilized antibodies to rapidly and accurately capture the analytes of interest (neuro-inflammatory biomarkers in complex biological fluids). It is postulated that with the ever increasing array of commercially-available antibodies, the chip-based system may be in the diagnosis of various NDDs.

Insulin degrading enzyme (IDE) is the main enzyme responsible for Aβ clearance from the brain [89]. IDE-mediated Aβ proteolysis is a progressive enzymatic process subjected to alternative substrate inhibition, especially by insulin. In another study, a CE method for *in vitro* investigation of IDE-mediated $A\beta_{1–40}$ proteolysis employing only a conventional CE instrument equipped with a fused silica capillary has also been reported [90]. It is hypothesized that further developments in this technique will be of major significance to biomedical utility in the future.

4. PROTEOMIC APPROACHES IN THE MANAGEMENT OF NDDs

High-throughput proteomics approaches are utilized to elucidate new therapeutic biomarkers which can be potential drug targets. While designing effective therapeutics for NDDs, rapid qualitative and quantitative analysis of neurotransmitters is a major concern. Vlcˇkova´ and Schwarz developed a quick but sensitive (3-8 times higher sensitivity) separation and detection method for catecholamines having similar structures such as noradrenaline, dopamine, adrenaline and their O-methoxylated metabolites including 3-methoxytyramine, normetanephrine and metanephrine from the mouse brain homogenate using a complex phosphate-borate buffer with sodium dodecyl sulfate (SDS) and polyamidoamine dendrimer and by using CNT-film-modified gold electrode detector [91]. Overall, carbon nanotube-based detectors have been applied in microchip and CE systems for the detection of neurotransmitters by several group of researchers [91-93].

In addition, the presence of $A\beta_{(1-40)}$ and $A\beta_{(1-42)}$ in the cerebrospinal fluid and plasma have been proposed as potential biomarkers of AD [94]. Therefore, quantification of circulating Aβ peptides in plasma can facilitate AD diagnosis. To determine the most suitable technique for detecting $A\beta_{1-40}$ levels in plasma or serum, Varesio *et al* compared NLC and CE attached to mass spectrometers [95]. A 50 μm I.D. CE capillary and a 75 μm I.D. NLC column were coupled to a single quadrupole mass spectrometer with a sheath-liquid electrospray interface and a nanospray interface, respectively [95]. It was concluded that CE has comparatively lower sensitivity, thus limiting its usage in biological matrix analyses. On the other hand, NLC has column switching set-up and higher sample loading capability, thus allowing better sensitivity of detection of $A\beta_{1-40}$ when used at ng/ml concentrations [95].

For effective treatment of NDDs, the levels of fluid $A\beta_{1-42}$, tau and phosphorylated tau can be good indicators [96]. Blood-brain barrier (BBB) acts as the biggest hurdle in the delivery of therapeutic proteins to the CNS [97]. Brain microvessel endothelial cells (BMVEC) which forms tight extracellular junctions and have low pinocytic activity are mainly responsible for the low permeability seen with the BBB [24]. Therefore, the development of new innovative methods and techniques for effective delivery of drugs and bio-macromolecules to the CNS is the primary requirement for the treatment of NDDs [98].

A plethora of methods and techniques are also applied in protein modifications so as to enhance penetration into the BBB. For this purpose, proteins have been conjugated with wheat germ agglutinin [99, 100], cationic compounds [101-103] and transferrin receptors [104-107]. In addition, antibodies were also conjugated with insulin for similar function. Even though the above mentioned strategies are rather successful in increasing the uptake of proteins into the CNS via adsorptive endocytosis, various aspects such as toxicity and antigenicity of such modified proteins can pose as limiting factors [104]. In cationization process, superficial carboxyl groups present in the protein is converted into an extended primary amino group which can be used to enhance the interaction of modified proteins with negative charge substances at the luminal plasma membrane of the brain endothelial cells. The cationized protein then undergoes adsorptive transcytosis occurring via the BBB.

Cationization of antibodies can be achieved by using several synthetic (hexamethylenediamine) or naturally occurring (e.g., putrescine) polyamines with the later being the most efficient. Nevertheless, although this approach is not free from some drawbacks, it is still a realistic approach for transportation of antibodies across the BBB [101-103]. In another successful example of conjugation, the OX26 antibody was conjugated with basic fibroblast growth factor and brain-derived neurotrophic factor and showed promising results in cerebral trauma models [108-110].

Protein transduction domain (PTD) is another significant approach for cellular delivery of polypeptides, polynucleotides and NPs. PTDs are small cationic peptides which can facilitate the uptake of large, biologically-active molecules into mammalian cells. They are significant because they can eliminate the problems posed by size restriction shown by some useful but otherwise larger drug molecules, thus enabling previously unavailable drugs to modulate and alleviate several diseases [111, 112]. Several sources of PTDs have also been explored including human immunodeficiency virus-1-transcriptional activator (HIV-1-TAT) peptide, DNA-binding protein (VP22), *Drosophila Antennapedia* (Antp) homeotic transcription factor and herpes simplex virus-1 (HSV-1). The HIV-1-TAT peptide is a small basic peptide successfully shown to deliver a large variety of molecules including small particles, proteins, peptides and nucleic acids. However, the region displaying properties of good cell penetration appears to be confined only to a small stretch (RKKRRQRRR) of 9 basic amino acids [113]. PTDs can be used in several ways. They can be introduced into protein by chemical conjugation method or alternatively, can be genetically-fused to the protein cDNA and expressed in host mammalian cells via transfection or they can also be produced in bacteria even though the mechanism of transduction still remains unknown [112, 114, 115]. To date, various TAT fusion proteins have been investigated for the treatment of NDDs [116]. Some successful examples include the intravenous delivery of TAT peptide conjugated with anti-apoptotic factor Bcl-XL and glial cells line derived neurotropic factor (GDNF), TAT-GDNF fusion protein in MPTP (1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine) model of AD [117-119]. Another PTD fusion protein conjugated with tyrosine hydroxylase showed significant effects on 6-hydroxydopamine induced PD model rats [120]. In other words, PTD conjugated protein modification strategy improves CNS drug delivery significantly, but its potential as treatment for NDDs is limited by its immunogenicity and long term side effects.

When compared to smaller molecules, the transport of biomacromolecules such as DNA or proteins across the BBB is more challenging. Nevertheless, naturally-occurring peptides which have the ability to effectively pass this barrier by receptor-mediated endocytosis (RME) provide some hope in this regard [121, 122]. The specific peptides can be used for targeting biomolecules by RME and can be selected from a phage display library [123]. Conjugation of these peptides with an appropriate drug carrier molecule further facilitate its transport [124]. However, limitation in size not exceeding 100 nm is the primary requirement for carrier molecules. Besides the above, NPs can effectively be used as a vehicle for drug and gene deliveries [125-127]. Galectins can also be conjugated with NPs as they have been reported to have significant roles in NDDs by virtue of their wide spectrum of properties [128-130]. Other examples include solid NPs [126, 131, 132], liposomes [133, 134] and polymer micelles [135-138]. The modification of carrier by polyethylene glycol (PEG) can be carried out to improve the stability of NPs in dispersion and increase their bioavailability [139-142]. Peptides and proteins can be attached to the end of PEG chains to facilitate receptor-mediated bindings. In addition, antibody and insulin-conjugated micelles have been found to be very effective in *in vivo* drug delivery to the brain tissue [143]. Immunoliposomes carrying therapeutic antisense epidermal growth factor receptor (EGFR) gene have been reported to deliver substances to EGFR-dependent brain gliomas *in vivo* successfully [144]. PEGylated immune-liposomes containing antibodies directed to insulin or transferrin receptors have been successful as carriers for gene replacement in PD model [145]. The PEGylated immune-liposomes are also used for targeting and transfecting β-galactosidase (LacZ) and luciferase into the brain [146, 147]. In another exciting strategy, a new family of carrier system called nanogel has been introduced for specific targeting of drugs and biomolecules to the brain [148]. Nanogels are cross-linked polymers often made by a combination of ionic and non-ionic polymer chains and are prepared following emulsification using a solvent evaporation system [149, 150]. Nanogels tend to swell in the presence of water and can incorporate several biomolecules including the oligos, siRNA, proteins, DNA and drug molecules with an encapsulation efficiency of approximately 40-60%, and they also have the ability to decrease possible degradation of biomolecules occurring during transportation [151]. Nanogels can also interact with a large number of biomolecules such as negatively-charged peptides and proteins. The surface of nanogels can be modified in many ways by using either transferrin or insulin by avidin-biotin coupling or for targeting receptors present at BMVEC.

For the treatment of NDDs such as AD and PD [152, 153], lysosomal diseases [154, 155] as well as obesity [121, 156], there is an urgent need to augment delivery of therapeutic peptide and proteins to the brain, besides using them as targeted moieties [152, 153]. RME (such as insulin, insulin-like growth factor and transferrin) is utilized by known peptides in order to cross the BBB to a remarkable extent [157]. For example, in artificial hydrophobization, a very small number of fatty acid residues (stearic, palmitic and oleic acid) can be conjugated with protein molecules [158-161]. In this process, a protein molecule is altered in a system of reversed micelles due to the presence of water-insoluble reagent such as fatty acid chloride. Fatty-acylated proteins then acquire the ability to translocate across plasma membranes and penetrate into intact cells. Protein conjugation with controlled modification can be achieved by using reversed micelles, resulting in an increased binding of these proteins with the lipid membranes as a result of anchoring of the hydrophobic groups [157]. With this approach, the protein molecule acquires hydrophobic anchor groups which can specifically target hydrophilic proteins to the cell surfaces besides remaining to be water soluble. By utilizing this technique, more than a dozen of proteins have been modified with their functional activities remaining intact [161-168]. Insulin modified with a palmitic acid residue is a successful example of enhanced hypoglycemic effect generation since it was less immunoreactive when compared to the native insulin [160]. Chekhonin *et al* reported that the interaction between fatty acid residue and antigen binding site is vital for the delivery of modified antibodies to the brain [165, 169]. A group of researchers in France synthesized a fatty acylated ribonuclease A (Rnase A) with the ability to cross BMVEC monolayer with minor degradation [170]. Furthermore, stearoylation of Rnase A [170] or horseradish peroxidase [171] significantly increases their penetration across the BBB. Amongst the fatty acid derivatives, stearic acid was found to be most active [170]. Protein conjugation with Pluronic® block copolymers is another successful strat-

egy/approach to successful delivery of drugs and biomolecules across the CNS [171]. The block copolymers are composed of hydrophilic ethylene oxide and hydrophobic propylene oxide arranged in a particular pattern which can thus interact simultaneously with hydrophobic surfaces as well as the plasma membrane due to their amphipathic nature. Their mode of action includes inhibition of drug efflux transporters expressed in BBB, thus allowing increased transport of various substances to the brain [172, 173]. Overall, the above approaches are very promising for improving drug delivery of protein-based diagnostic and therapeutic agents to the CNS. A schematic representation of nanotechnology-based drug delivery approaches in the treatment of NDDs is depicted in (Fig. **3**).

5. NANOTECHNOLOGICAL APPROACHES IN THE MANAGEMENT OF NDDs

The mysteries associated with various NDDs may be unraveled by newly emerging nanotechnological approaches, since some of the methods have been reported to correlate with NP exposures. A positive result in this aspect shows that the damaging effect of human exposure to toxic NPs can be reduced by identifying creation-exposure pathways of toxins [174]. On the other hand, the toxic properties of some NPs may allow treatment of diseases at cellular level and could potentially be utilized for the treatment of various NDDs.

Among currently available therapies for various NDDs, the oral administration of dopamine agonists such as levodopa is the most common. However, the effects of levodopa along with the motor side-effects tend to decline among NDD patients with disease progression. Therefore, transplantation of fetal dopamine neurons and deep brain stimulation has been explored to complement the pharmacological treatments [175, 176]. Nevertheless, these approaches still remain inconclusive and are not very effective in halting the continual loss of dopamine neurons [177, 178] thus creating the need of new approaches to combat the progression of NDDs.

Nanotechnological approach can be a potential way towards further understanding and management of various NDDs. In this regards, a number of nanostructures have been employed for the development of nano-enabled drug delivery. Some of the nanostructures already in use include polymeric Nps [179], polymeric nanospheres and nano-suspensions [180], polymeric nano-gels [150], carbon nano-tubes and nano-fibers [44], polymeric nano-micelles [181] and as well as polymeric nano-liposomes [44]. Even though these approaches have shown some promising results, more concrete scientific efforts remain desired. Table **1** lists the various types of nanostructures used in the treatment of NDDs.

6. METABOLISM AND DISPOSITION OF NANO-DRUGS

Nano-drugs are the result of the interplay between nanotechnology and modern medicine (nanomedicine). Nano-drugs adsorb NPs on their surface and are specifically targeted to a particular cell or organ to provide maximum safety with minimal side effects [182-184]. Nano-drugs have the advantage of overcoming the natural barriers present in our body defense system which generally can pose several challenges to drug delivery. Nano-drugs can enter the capillaries, penetrate cells, get absorbed though pinocytosis and can enhance bioavailability [97]. Due to their large surface area, nano-drugs can further enhance the solubility of poorly soluble drugs and also increase their half-lives by controlling the speed of degradation *in vivo*, thus augmenting drugs' efficacy and lowering their side effects [184-187]. After entering the body, drug and NPs get separated under a constant speed thus creating a time lapse before they reach their targets [188]. Nano-drugs have been reported to reach into specific body parts mainly by infiltration, leaching and proliferation (dissolution) [189]. The conjugation of drugs with NPs prevents enzymatic degradation of drugs, increases effective drug release time, reduces side effects and improves efficiency.

Some of the effective methods of controlled nano-drugs release include chemical, solvent and diffusion control [190]. Among these, diffusion control is the most commonly used method for nondegradable polymeric carriers [191-193]. For the chemical control method, hydrolysis and other types of chemical reactions efficiently reduces time and rate of drug release [194]. Depending upon the role of drugs and NPs involved, chemical control method utilizes two different approaches; the side-chain and the degradable systems [97]. The rate of polymer degradation by different enzymes is the limiting factor for nano-drug release rate in the degradable system [195]. Following degradation, the metabolites can be absorbed or discharged by the body, thus regulating polymer degradation at a particular location within a regular time frame [196]. The limiting factor is the polymer degradation rate which not only affects the release of nano-drugs in a specified time, but is also

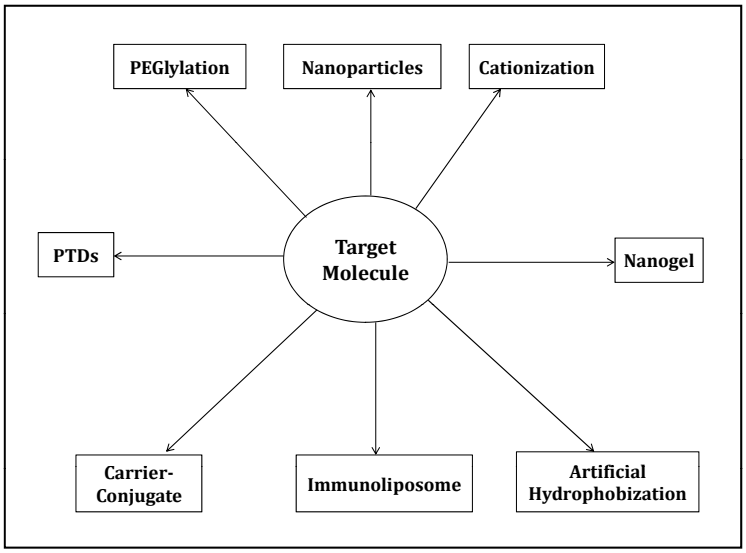

Fig. (3). Schematic representation of nanotechnology-based drug delivery approaches in the treatment of various NPs.

Table 1. Nanostructures used in treatment of neurodegenerative disorders. (Aβ, amyloid-β; AD, Alzheimer's Disease; Ach, Acetylcholine; NGF, Nerve Growth Factor; PD, Parkinson's Disease).

Nanostructure	Target	Drug Delivered	References
Nanocapsules & nanospheres	Neuroinflammation	Indomethacin	[209, 210]
	Aβ in AD	Thioflavin-T	[211]
	Aβ in AD	Clioquinol	[212]
	Aβ in AD	d-Penicillamine	[213]
Nanogels	Brain delivery	Oligonucleotides	[149, 151]
	Aβ in AD	Cholesterol bearing pullunan	[209]
Carbon nanotubes & nanofibers	Nerve growth	NGF	[214]
	Dopamine in PD	Biosensor	[214]
	AD	Ach	[215]
Nanomicelles	Aβ in AD	PEGylated phospholipids	[216]
Nanoliposomes	Aβ in AD	Curcumin	[217]

dependent on other factors such as quality of the polymer, its molecular weight, crystalline nature of NPs, hydrophilicity and hydrophobicity [197]. In the side-chain release method, both degradable and nondegradable types of nano-drug carrier can be used. The side chain which is attached via some chemical bonds can be broken by hydrolysis or enzymes, thereby controlling the release of drugs [197]. Beside these factors, the release of nano-drugs is affected by several other parameters, such as the nature and composition of polymers and nano-drugs, surrounding temperature and environment, pH, and hydrophilicity and hydrophobicity of degradable polymers.

A major concern with the use of nano-drugs is the evaluation of their biosafety and toxicity [198-202] profiles. To date, there are only few studies related to the toxicity of NPs that are in use [203-205]. Overall pharmacodynamics, tissue distribution, plasma clearance and urinary excretion of nano-drugs still need some careful evaluation [206]. Nanomaterials consist of metal components like quantum dots, nano-gold, nano-silver and nano-zinc oxide. When these metal-based nanomaterials are used for biological applications, their biosafety must be monitored. Furthermore, the biological disposition including the ADMET (absorption, deposition, metabolism, elimination and toxicity) of the nanomaterials needs to be further evaluated. Such evaluation can be done by tracking the changes in the metallic constituents of NPs in various tissues and organs following exposure. Atomic absorption spectrometry (AAS) and inductively-coupled plasma mass spectrometry (ICP-MS) are the preferred techniques for metal analyses [207].

Four mechanisms of NP toxicity have been identified: 1) toxicity of any constituents present 2) toxicity of their degradation products 3) toxicity as a result of endocytosis of NPs and 4) toxicity-mediated membrane lysis [196]. The U.S. Food and Drug Administration (FDA) have developed a new task force on nanotechnology, but more insight or regulation needs to be practiced. Researchers from all over the world have expressed their anxiety about the harmful effects of nano-drugs on human health [196]. This is due to the fact that the minute size of NPs theoretically can allow their infiltration into all body cells and can therefore be potentially detrimental to healthy cells. Another vital concern is the proper disposal of NPs used in the manufacturing or other processes. Special disposal techniques are required to avoid harmful particles from accumulating in the environment following which monitoring may be an uphill task if not impossible [208].

7. CONCLUSION

The limited available therapeutic options for NDDs have extended some new therapeutic prospects. Modern nanotechnological approaches coupled with new proteomic tools promise to be a revolutionizing way of further understanding and managing these disorders. With the recent advent of carbon nanotube-based detectors, rapid qualitative and quantitative analyses of neurotransmitters are feasible. One good example is the excellent NP-based system as drug (nano-drugs) and gene delivery vehicles to penetrate the unique BBB layer. These are rather promising in exploring novel approaches in early protein-based diagnostics focusing on the detection of pre-inflammatory states and plaque characterization as well as new therapeutic agents. Reducing toxicity and increasing target specificity of nano-drugs will hopefully transform numerous facets of brain physiological studies and clinical neurology in the future.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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LIST OF ABBREVIATIONS

NDD = Neurodegenerative disorder

AD = Alzheimer's disease

PD = Parkinson's disease

Aβ	=	Amyloid-β
BBB	=	Blood-brain barrier
CNS	=	Central nervous system
NFTs	=	Neurofibrillary tangles
APP	=	Amyloid precursor protein
PINK1	=	PTEN-induced kinase
UCH-L1	=	Ubiquitin carboxy-terminal hydrolase L1
IDE	=	Insulin degrading enzyme
NPs	=	Nanoparticles
NLC	=	Nano liquid chromatography
NHPLC	=	Nano high performance liquid chromatography
NCE	=	Nano capillary electrophoresis
CE	=	Capillary electrophoresis
PTD	=	Protein transduction domain
PEG	=	Polyethylene glycol
BMVEC	=	Brain microvessel endothelial cells
RME	=	Receptor-mediated endocytosis

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