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Review

Nanotechnology platforms in Parkinson's Disease

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Abstract

Parkinson's disease (PD) remains a serious concern due to its effects on the quality of life of patients and its socioeconomic burden to society. Present day management of PD has limitations in both diagnosis and treatment. Nanotechnology may provide smart solutions to this problem. The present review highlights the recent advancements in the development of nanotechnology platforms for PD. The review focuses on the use of such platforms in diagnostics, treatments, deep brain stimulation, neurosurgery and other modalities of management and the role of nanotechnology in each of these fields. The review also sheds light on the translation of technologies from labs to clinics and the essential advantages as well as concerns that accompany the translation.

Keywords

Smart materials; nanoparticles; sensors; nanotechnology; drug delivery; blood brain barrier.

Introduction

Parkinson's disease (PD), in the present day, ranks second after Alzheimer's disease among the most common neurodegenerative diseases and has characteristic motor and non-motor symptoms [1]. Globally, the prevalence of PD has been found to be close to 0.3 % of the total population and the crude incidence rate is 4.5–19 per 1,00,000 population per year [2,3]. The World Health Organization computed the disability-adjusted life years (DALYs) or the number of years lost of healthy life, due to PD to be 1,617,000 globally in 2005 and projected it to increase to 2,015,000 years by 2030 [3]. It would mean that more and more people would be leading lives with disabilities due to Parkinson's disease. Besides, there is a huge cost that is incurred globally for the management of PD. In developed countries like the United States, the national economic burden of PD was more than USD 14.4 billion in 2010 (approximately USD 22,800 per patient) [4]. In developing countries like India, patients with PD were found to spend nearly 16 % to 41.7 % of the average Indian gross national income as direct costs in the treatment of PD [5]. Thus, PD is a universal disorder and in addition to the clinical features, the socioeconomic impact of PD is a cause of concern.

The management of PD has several limitations at present. The clinical diagnosis of PD originates to the

acclaimed neurologists like Parkinson and Charcot who observed that the presence of two or three motor features including bradykinesia, rigidity and tremor was characteristic of Parkinson's disease [6]. Two centuries later, the issue with the clinical diagnosis of Parkinson's disease today is that it fails to differentiate it from the many other forms of parkinsonism [7]. Also, it requires expert clinicians for the evaluation of these subjective features of the disease. Thus, there is a need for better biomarkers that can provide for an objective assessment of the presence or absence of PD. Beyond the diagnosis, standard treatment modalities comprise of therapy using dopamine agonists and dopamine precursors to improve clinical symptoms associated with disease progression, patient's care and quality of life. However, short half-lives, treatment intolerance together with side effects like levodopa (L-3,4-dihydroxyphenylalanine or L-DOPA) induced dyskinesias limit the long term treatment efficacy. Further, therapy is limited by the presence of the "blood-brain-barrier". Thus, in the sphere of treatment for PD, there is a need to develop efficient therapies with minimal side-effects and increased bioavailability in the central nervous system.

Nanotechnology, an emerging tool, has the potential to improve these circumstances by introducing novel carrier-based platforms that will target selective release of drug payload with on-demand and controlled release kinetics and increased reach via modulating or by-passing the blood-brain-barrier. The present review describes the use of nanotechnology-based platforms for diagnosis, drug delivery and other therapeutic approaches.

Epidemiology of Parkinson's Disease

Prospective and well-designed population based studies reported that prevalence and incidence rates of PD were age-specific *i.e.* onset of disease is rare below 50 years of age whereas sharp rise (~1-1.5 %) is seen over 60 year of age resulting in prevalence less than 0.04 % below the age 40 years, which rises to 19 % above the age of 80 years [2,8]. The prevalence of PD varies by gender (men are more prone to PD than female, possibly due to the neuroprotective effect of estrogens), race/ ethnicity (incidence rates were found to be highest among Hispanics>non-Hispanic>Whites>Asian>Blacks) [9]. Differences in environmental factors like occupational exposures (pesticides, heavy metals) and cigarette smoking; dietary factors (anti-oxidants like vitamin E, fatty acids mainly unsaturated fatty acids, dietary iron etc.), genetic factors like α-synuclein (PARK1), parkin (PARK2), genes involved in dopamine metabolism, homocysteine metabolism and polymorphism in mitochondrial DNA etc. are responsible for PD progression with variations in its prevalence and incidence [9].

Etiology and Pathophysiological Hallmarks of Parkinson's Disease

For designing of successful diagnostic and therapeutic strategies against PD it is important to understand the etiology and pathophysiology behind development and progression of PD. PD is a chronic, progressive neurodegenerative disease characterized by impairment in motor and non-motor activities with symptoms like akinesia, tremor, muscle rigidity, postural instability, freezing of gait and depression, delirium, dementia, sleep disorders, autonomic symptoms (bradycardia, sialorrhea, sexual dysfunctions and dry eyes syndromes etc.) and other symptoms like olfactory disturbances, fatigue, weight changes etc. The classical pathophysiological hallmark of PD is the presence of Lewy bodies in the dopaminergic neurons that are left behind after degeneration of these neurons in substantia nigra pars compacta. This results in dopamine depletion in the striatum and basal ganglia nuclei due to innervations of dopamine neurons in two segments of globus pallidus i.e. external and internal parts of basal ganglia nuclei and also auxiliary to subthalamic nucleus and thalamus [10]. The etiological factors include toxins mainly MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) which produced MPP+ (1-methyl-4-phenylpyridinium), a

neuro-toxic precursor by monoamine oxidase-B (MAO-B) that further interferes in mitochondrial metabolism leading to free radical generation and subsequently causes dopaminergic neuron degeneration in the substantia nigra [11]. Various other toxins include TaClo (formed from interaction between sedative agents like chloral or organic solvents like trichloroethylene with endogenous tryptamine) were also reported to be toxic to dopaminergic neurons [12]. Other pathological hallmarks includes degeneration of non-dopaminergic neurons leading to depletion of nor-adrenaline, serotonin (depletion or hyperactivity of 5-hydroxy tryptamine in the brain giving rise to symptoms such as depression and hallucinations), glutamic acid, gamma-amino butyric acid (GABA), neuropeptides like endorphins (enkephalins and dynorphins) and acetylcholine etc. [13-16].

The pathophysiology of PD remains ambiguous, and is under continuous active investigations till date. Olanow and Tatton (1999) have reported that potential genes like glutathione peroxidase, superoxide dismutase-1, 2 and dopamine receptors (D2-4) etc. contribute to 5-10 % familial PD with autosomal dominant or recessive inheritance [17]. The pathogenesis of PD includes oxidative stress in substantia nigra through production of reactive oxygen species (ROS) and hydrogen peroxide by extensive oxidative metabolism of dopamine. Collectively, the increased turnover of dopamine, decreased glutathione levels and presence of reactive iron promotes generation of free radicals which may be responsible of disease development and progression [17]. Previous studies reported oxidative damage to the DNA {elevated levels of 8-hydroxyguanine (8-OHG)}, in substantia nigra of PD patients due to free species like peroxynitrite, hydroxyl radicals etc. [18]. In 1990, Schapira and co-workers reported the role of increased mitochondrial dysfunction in PD patients and association with defects in mitochondrial complex-I of the respiratory chain leading to nigrostriatal neuronal degeneration through depletion of ATP synthesis [19]. Several studies demonstrated that different genes like α-synuclein, parkin and UCH-L1 (gene encoding ubiquitin carboxyl-terminal hydrolase L1) act concomitant with an increased mitochondrial dysfunction and oxidative damage in PD patients [20]. Song *et al.* (2004) reported a co-relation between α-synuclein and mitochondrial function, since α-synuclein transgenic mice were susceptible to neurotoxic effects induced by MPTP whereas, resistance was observed in α-synuclein knockout mice [20]. Sheng *et al.* reported the up-regulation or mutation in α-synuclein leads to formation of abnormal and misfolded proteins through series of events like oligomerization, fibrilization and aggregation within the nerve cells (formation of Lewy bodies) that responsible for mitochondrial dysfunction, impaired neuronal homeostasis and lead to subsequent neurodegeneration [21]. Several studies reported the role of genetic factors in pathogenesis of PD such as extensive generation of Lewy bodies one of the pathophysiological hallmarks associated with mutated α-synuclein causing early and late onset of PD with rapid disease progression whereas, mutated LRRK2 showed late onset of PD (at 80 years of age) with Lewy bodies formation, however dementia was not observed and also mutated ATP13A2 cause an atypical PD with dementia [22-24]. Another study demonstrated that activated macrophages and microglia responsible for secretion of different neurotrophic factors like brain derived neurotrophic factor (BDNF), and glial derived neurotrophic factor (GDNF) cause dopaminergic sprouting in nigrostriatal injury in PD patients [25].

Paul and colleagues reported the significant role of ubiquitin–proteasome system (UPS) and autophagy (macroautophagy) pathways in lysosomal and proteasomal dysfunction, another pathophysiological hallmark of PD [26]. Further studies reported the damage to UPS and lysosomal pathways (increased protein accumulation due to impaired unwanted protein clearance mechanism) through mutated genes like Parkin, LRRK2 causing neuronal toxic insult in PD [27].

Impaired iron metabolism concomitant with different genetic (α-synuclein aggregation) and environmental (tobacco, cigarette smoking) factors was observed in pathogenesis of sporadic or familial PD [28]. Mitochondrial byproducts (peroxide and superoxide) can reactive with iron by generating most damaging hydroxyl radicals causing dopaminergic cell death in substantia nigra. These findings further supported by chronic MPTP treated mouse studies correlating iron levels with selective dopamine degeneration through lipofuscin aggregation and up-regulated α-synuclein [29]. PLA2G6 and ATP13A2 are two important genetic factors associated with increased neurodegeneration in brain. Iron accumulating in substantia nigra of PD patients have been reported by several studies however their precise mechanism of action is not yet clear [30,31]. Despite the significant progress in molecular and cellular studies, the detailed pathophysiology of PD is not yet clear.

Present modalities of management and their limitations

Current Techniques for Diagnosis of PD- Clinical Diagnosis and Imaging

Accurate diagnosis plays an important role in the precise management of PD. Currently, diagnosis of PD is done on the basis of different clinical symptoms of parkinsonism like bradykinesia, tremor, postural imbalance, dementia, sleep disorders, sialorrhea, dry eyes syndromes and olfactory disturbances, fatigue, weight changes [32]. Presence of Lewy bodies in substantia nigra, hypothalamus, basal nuclei, and cranial motor nerve and its nuclei etc. is an important biomarker for PD diagnosis. Different genetic and imaging testing were carried out for diagnosis of PD. Identification of genetic mutations revealed that α-synuclein, NURR1, LRRK2 and UCH-L1 are responsible for autosomal-dominant inheritance whereas, DJ1, Parkin and PINK1 are responsible for autosomal-recessive inheritance [32]. The cranial CT scan, MRI and PET (fluorine-18 labeled DOPA) imaging, SPECT (single photon-emission computed tomography) and transcranial ultrasound imaging etc. are valuable techniques for differentiation and diagnosis of PD from other neurodegenerative disorders. For instance, SPECT tracer used for presynaptic dopamine transporter imaging like FP-CIT (*N*-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) nortropane binds to the dopamine transporters (DAT) protein. Thus, integrity of nigrostriatal dopaminergic transporter can be used as a diagnostic marker for detection of early PD progression [33]. However, accessibility and cost of these techniques restrict their usefulness and application in diagnosis of PD.

Pharmacotherapy and Management of Parkinson's Disease

Currently different therapies are available for treatment of motor and non-motor complications associated with PD. Pharmacotherapy of PD includes treatment with drugs like L-DOPA, dopamine receptor agonist, catechol-O-methyltransferase (COMT) inhibitors, antagonists of MAO-B and glutamate and amantadine etc. L-DOPA is most widely used therapy for treatment of PD because it easily crosses BBB and is converted into dopamine in presence of an enzyme DOPA-decarboxylase. However, long term treatment with L-DOPA results in various side effects like dyskinesia (involuntary and non-rhythmic choreic or choreo-dystonic movements), alterations in on-off periods giving rise to motor fluctuations, nausea and vomiting [34]. Therefore, in clinical practice L-DOPA is co-administered with Carbidopa (DOPA-decarboxylase inhibitor) in which Carbidopa itself does not cross BBB but reduces conversion of L-DOPA to dopamine in peripheral nervous system and thereby increase its delivery to the brain [35]. COMT inhibitors (entacapone) are ineffective when administered alone, therefore they are administered as adjuvants with Levodopa and Carbidopa in PD patients to avoid end-off-dose alterations [36]. Early PD symptoms are treated with MAO-B inhibitors like selegiline or rasagiline. Recently, Schapira *et al.* (2013) reported the dual action of safinamide (selective and reversible MAO-B inhibitors that decrease glutamate release *via*

blocking voltage depended sodium channels) by preventing dyskinesia and motor fluctuations in early PD patients [37]. Further randomized and double blind phase II/III studies demonstrated the anti-parkinson efficacy of safinamide therapy in PD patients [38]. Dopamine (post-synaptic D2 receptors) agonists such as non-ergot derivatives (pramipexole, ropinirole and rotigotine) are also used in early PD patients (above age of 60 years). However, treatment with dopamine agonists is not free from side effects like confusion, hallucinations and psychosis etc. Recent studies described the role of Adenosine A2A receptor antagonists in treatment of PD through increased activation of dopamine (D2) receptors via inhibiting adenosine receptors in striatum and basal ganglia nuclei. For instance, adenosine A2A receptor antagonist (Istradefylline) an adjuvant with levodopa has significant potential to prevent levodopa induced off-time fluctuation and dyskinesia [39]. Glutamate, an excitatory neurotransmitter in brain, has a significant role in pathogenesis of PD; hence glutamate receptors (subtypes of glutamate receptors like N-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)) antagonists can be co-administered with levodopa to alleviate dyskinesia and other motor symptoms. Recent clinical trials with AFQ056 (mGluR5 i.e. metabotropic glutamic receptor antagonist) demonstrated an anti-dyskinetic therapy with improvement in motor symptoms. However, glutamate receptor antagonists are associated with various side effects like fatigue, and gastrointestinal symptoms [40].

Different antioxidants (Coenzyme Q10 and N-acetyl-cysteine) that scavenge reactive oxygen species may have potential in preventing oxidative stress and dopaminergic neuronal degeneration in the nigrostriatal pathway in PD [41]. Neuroprotective therapies include use of various neurotropic factors like GDNF may induce dopamine differentiation/regeneration, and improved motor functions [42]. However, unsatisfactory pre-clinical outcomes were observed since neurotropic factors have limited BBB permeability when administered systemically. Their direct administration through intraputaminal implantation into basal ganglia has been reported in a randomized clinical trial by Lang and colleagues [43]. However, patient-friendly and non-invasive approaches need to be developed for effective and safe neurotropic therapy in PD in the future [43].

Neuronal signaling in the central nervous system occurs through a combination of different electrical and chemical signals which maintain the homeostasis by precise regulation of ionic composition across the system. However, this neuronal regulation is defined by presence of barrier layers across the system. There are three different types of barriers at the interface of blood and the central nervous system i.e. blood-brain-barrier (BBB), blood-cerebrospinal fluid-barrier (BCSFB) and the arachnoids barrier. The BBB is widely studied as it is the major limiting factor for exchange between blood and brain. BBB with average surface area of about 200cm^2 g-1 is formed by the brain microvascular capillary endothelial cells (BMVEC) comprising tight junctions (TJs) formed by proteins like occludins, claudins, zonula occludens etc., adherent junctions (AJ) proteins like catenins, cadherins; and junctional adhesion molecules [44], astrocytes (BBB endothelium enclosing glial cells), basement membrane and pericytes (undifferentiated connective tissue cells that promote proliferation, migration and differentiation of endothelial cells). Together these structural components play a dynamic role in maintaining brain homeostasis, barrier function and integrity of the BBB [45]. Tight junctions maintain high transepithelial electrical resistance (1500-2000Ω cm^2 over other peripheral microvessels) which shields the brain from different neurotoxins. The BMVEC lacks fenestrations and is reported to have a higher mitochondrial load as compared to other endothelial cells consequently playing an important role in the active transport of nutrients to the brain [46]. Different efflux transporters (P-glycoprotein, multidrug resistance associated proteins), enzymes (γ-glutamyl transpeptidase, alkaline phosphatase) and receptors (low density lipoproteins receptor, epidermal growth factor receptor) are expressed on either luminal or abluminal membrane surfaces of BBB to prevent

transport of substances across the barrier [47,48]. The high electrical resistance of tight junctions restricts the entry of macromolecules whereas ions and other polar compounds can only access the BBB via paracellular diffusion. There are various specific and selective pathways for transport of different nutrient molecules across BBB (like solute carriers, transporters for glucose, amino acids etc. However, presence of efflux transporters like P-gp and tight junctions within the BBB are the additional challenges for effective drug delivery to the CNS [49,50].

Standard pharmacotherapy for PD has a poor therapeutic efficacy, with only a symptomatic relief i.e. they do not modify or identify the progress or root cause of the disease and show treatment failure often associated with various side effects. The limitations of current therapeutic modalities, as outlined in Fig. 1 need to be addressed for the appropriate treatment of PD.

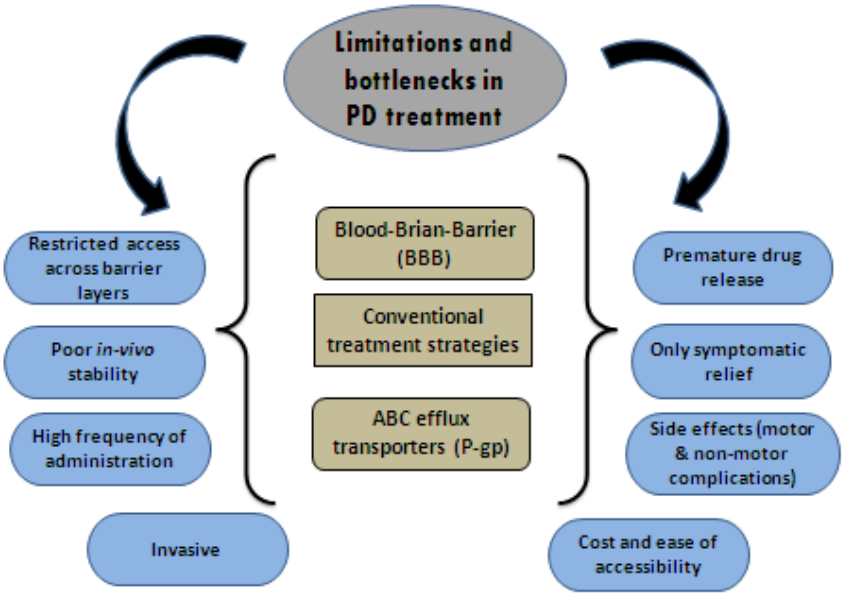

Figure 1. Limitations in the treatment of Parkinson's disease

Neurostimulation by Surgical Approaches

Different surgical approaches have been under clinical practices for the management of PD. Deep brain stimulation is one of the surgical approaches that involve implantation of CT/MRI guided electrodes in the subthalamic nucleus or in the globus pallidus. The impulse generator creates electrical stimulation affecting the firing pattern of neurons in the implanted area along with the release of neurotransmitters glutamate and adenosine and improves the motor symptoms in PD with neurogenesis in addition to causing an increased blood circulation in the stimulated regions [51]. Several studies have reported that deep brain stimulation as an adjuvant with pharmacotherapy showed significant improvements in patient daily activities and quality of life by reducing motor disability, levodopa induced motor fluctuations [52]. However, it is often associated with many adverse effects like intracranial hemorrhage, cognitive impairment, anxiety, infection and post-operative seizures. [53,54]. Thalamotomy, is an invasive approach performed using a probe in region contralateral to the tremor and lesion in severe PD cases. Recently transcranial MRI guided focus ultrasound technology was use to perform thalamotomy which significantly reduced tremor, postural disability and improved the quality of life over a period of one year post-treatment. However, side effects include cognitive impairment, paresthesia of tongue and figure, headache and nausea [55]. Gamma knife (GK) is another non-invasive surgical procedure where the emitted gamma

radiations intersect within the target brain region within 15-30min under anesthesia and led to a significant improvement in tremor and daily activities after several months of treatment. However, GK procedure suffers from lack of target region accuracy hence demands guided assist for more precise neurostimulation against PD [56].

Nanotechnology as a viable alternative

Nanotechnology and nanoscience are the interdisciplinary areas involving the development of nanosized materials. The foundations of these areas and the concept of miniaturization involved were introduced by Richard Feynman half a century ago in his renowned talk titled: "*There's plenty of room at the bottom."* [58]. Principally, nanoscience is directed toward manipulation of materials at atomic or sub-atomic levels whose properties differ significantly from those of bulk matter whereas nanotechnology aims to exploit such manipulated materials for designing, characterizing, and production of improved structure, devices and systems with controlled size and shape (1-100 nm) for various applications [57]. Nanotechnology has found numerous applications in biomedical sciences referred to as nanomedicine. For the purposes of nanomedicine, the definition of a nanomaterial is not restricted to those below 100 nm in size but extend to various submicron sized materials. The key aspect is to exploit the size dependant change in properties of submicron sized materials that can be exploited for altered cellular responses. Nanostructures have been applied for diagnosis of diseases, imaging, as devices, for tissue regeneration as well as for drug delivery and therapeutics. Nanotechnology which involves the manipulation of various systems at the nanoscale can have potential in the management of Parkinson's disease. A few advantages of nanotechnology platforms in PD are enlisted below:

1. Nanotechnology can help in the development of sensors that are able to sense various biomarkers at low concentrations in the presence of other analytes and help in the development of affordable diagnostic devices.
2. Nanotechnology-based drug delivery platforms play an important role to achieve better therapeutic efficacy and enhance bioavailability across BBB for the treatment of CNS disorders. The different platforms like active targeting using receptor mediated endo- or transcytosis, stimuli responsive nano-carriers and macrophage mediated passive targeting etc. are few of the approaches that can improve the treatment of PD.
3. Nanotechnology can also improve neural prosthetic devices for deep brain stimulation with better contact with the brain.
4. Nanotechnology can also help improve precision in neurosurgery through nano-level precision of Laser axotomies.
5. Lastly, nanotechnology may be employed to develop platforms for effective stem cell therapy through various tissue engineering approaches.

The role of nanotechnology in the development of smart platforms for the diagnosis and treatment in PD are discussed in the subsequent parts of the review.

Nanotechnology-based platforms for diagnosis

A brief outline of the technologies developed for the diagnosis of PD is shown in Figure 2. Nanotechnology is thus being infused into the *in vitro* diagnostics, novel biomarker identification and bioimaging modalities for the diagnosis of PD.

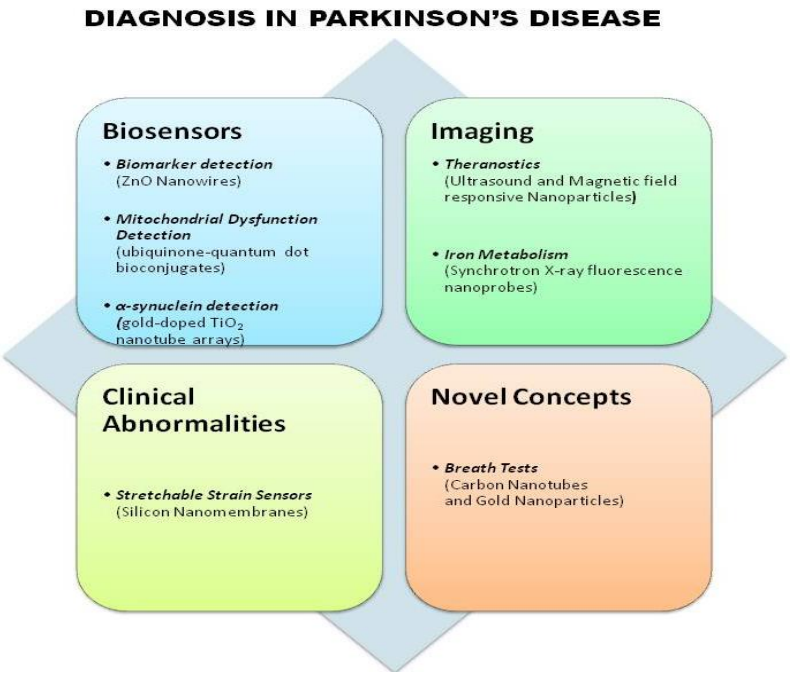

Figure 2. Use of Nanotechnology in diagnosis of Parkinson's Disease

Parkinson's disease faces the yet unmet need for development of well-established and validated biomarkers [58]. Hence, most of the present day end-points in the assessment of patient response in the clinics as well as in large clinical trials depend on subjective measures of patient response and scores obtained using scales like the Unified Parkinson's Disease Rating Scale [58]. As an improvement of the classical clinical features, these scales that included features like response to levodopa treatment along with motor evaluation were developed to improve accuracy of a clinical diagnosis [7]. However these scales still needed an expert clinician. In PD specifically, the clinical characters can be substantially masked due to compensatory mechanisms for many years and it is during this long prodromal period that there is further progression of Parkinson's disease. [7]. Hence, a framework needs to be developed for incorporating clinical knowledge, pathology, genetics and molecular mechanisms along with objective tests for defining the criteria for the diagnosis of Parkinson's disease [7]. This has led to effort like the Parkinson's Progression Markers Initiative (PPMI) funded by the Michael J. Fox Foundation as an effort to meet the unmet need of finding suitable biomarkers.

Various platforms to detect and quantify these biomarkers, PD-associated pathologies, imaging modalities and clinical abnormalities are being considered in the diagnosis of Parkinson's disease. A variety of nanoparticles manufactured from zinc oxide, carbon nanotubes, fullerenes etc. are described here which have the potential to be used in the diagnosis of PD.

Detection of biomarkers

The PPMI study is performing clinical tests on blood, urine and CSF samples; performing neuroimaging to identify better markers of the earlier and accurate diagnosis of PD and its progression [59]. The quantification of dopamine has been extensively used and has traditionally been estimated using spectrophotometry and chromatography.

Nanotechnology platforms for detection of dopamine: There are a number of nanotechnology-based approaches that simultaneously detect dopamine with other biomarkers. Tashkhourian *et al.* developed a modified carbon-paste electrode for simultaneous quantification of dopamine and ascorbic acid [60]. The electrodes were modified to incorporate silver nanoparticles and carbon nanotubes, which led to more

efficient electrochemical catalysis with high selectivity and sensitivity and a detection limit in micromolars for both the compounds [60]. In another study by Yue and colleagues, zinc oxide (ZnO) nanowire arrays (each nanowire being sub-100 nm in diameter and a couple of microns in height) were prepared upon a three dimensional foam made of graphene and the assembly could selectively detect dopamine along with urate and ascorbate by a method called differential pulse voltammetry. The addition of nanowires lead to high electrical conductivity and enhancement of sensitivity of electrochemical biosensors leading to reduction in the detection limit to 1 nanomolar for dopamine and uric acid in the serum of PD patients [61]. The field testing of this array lead to the interesting finding of a reduced UA level in 7 PD patients than in healthy individuals which can make uric acid a potential biomarker in the diagnosis of PD [61]. In yet another modification, Kurzatkowska *et al.* developed ion-channel mimetic self-assembled monolayers of macrocyclic polyamines deposited onto gold electrodes for electrochemical determination of dopamine [62]. The corrole molecules covalently attached on the surface of gold electrodes was an example of biomimicry of ligand-gated ion channels making the electrode surface semi-permeable to a redox marker. Upon addition of dopamine, a corrole-dopamine complex is formed on the monolayer leading to the recording of a positive charge on the electrode [62]. This allowed the detection limits to go down to the picomolar range [62]. Thus, the detection of dopamine can be made more sensitive and the detection limits can be reduced by orders of magnitude with the incorporation of nanotechnology-based smart platforms.

Mitochondrial dysfunction: As another example, PD has been associated with mitochondrial dysfunction and the inhibition of complex I comprising of NADH:ubiquinone oxidoreductase has been implicated in the demise of dopaminergic neurons [19]. Ma *et al.* developed ubiquinone-quantum dot (CdSe/ZnS) bioconjugates in which complex I and NADH could modulate the emission from the nanocomplexes by simulating the electron-transfer system part of the mitochondrial respiratory chain [63]. The authors showed that the bioconjugates could be used to monitor changes in fluorescence to trace complex I levels in human neuroblastoma SH-SY5Y cells [63]. Though in the proof of concept stage, this technique has the potential to serve as an *in vitro* biosensor for the early detection of PD and in monitoring disease progression.

α-synuclein detection: As described earlier, α-Synuclein is a very important neuronal protein associated with PD and present day strategies for its quantitative detection include sophisticated, time-consuming instruments including NMR spectroscopy, fluorescence measurements, western blotting and size-exclusion chromatography [64]. An *et al.* developed highly ordered microfabricated arrays using gold-doped TiO_2 nanotubes for photoelectrochemical detection of α-Synuclein [64]. The arrays were effective platforms for the immobilization of primary antibodies while retaining their stability and α-Synuclein binding [64]. Then, the attachment of secondary antibody and gold nanoparticle-conjugated glucose oxidase, allowed excellent sensitivity by signal amplification. Glucose oxidase catalyzed the conversion of glucose into gluconic acid and hydrogen peroxide. Upon irradiating the other side of the titanium foil, the holes that were formed within the valence band of the nanotubes could be scavenged by the peroxide leading to a photocurrent proportional to concentrations of α-Synuclein with a detection concentration in the range of pg/ml [64].

Other novel concepts: There are a few other novel diagnostic concepts that are emerging. The use of breath tests to diagnose neurodegenerative disorders is one such advancement. A number of volatile organic compounds like styrene, butylated hydroxytoluene and hexadecane have been found in the breath of PD patients [65]. As an example of the use of nanotechnology in the sensing of such novel compounds,

organically functionalized nanoparticles like carbon nanotubes and gold nanoparticles were used by Tisch and co-workers [66]. The sensors were effective in distinguishing the breath prints of PD from healthy states with an accuracy of 78 % [66]. Similarly, the technology could identify Alzheimer's disease and differentiate it from PD.

Improved imaging technologies

Imaging in PD is dependent on a number of sophisticated techniques like cranial CT, MRI, PET and SPECT which are mostly available at advanced healthcare centres and are expensive. A number of experimental approaches are being developed and have potential for the imaging and earlier diagnosis of PD. A few of these have been elaborated below.

Iron metabolism: Homeostasis of various metal ions which are found to be altered characterize neurodegeneration in PD. Ortega and colleagues used this for chemical nano-imaging *in vitro* using synchrotron X-ray fluorescence nanoprobe (88 nm beam) which allowed detection of 10^{-18} g of iron within cellular structures around 100 nm diameter [67]. Results suggested that PD showed elevation of iron in the substantia nigra pars compacta, with loss of tyrosine hydroxylase activity that may lead to reduced iron-dopamine binding making the dopaminergic neurons more prone to iron toxicity [67]. Thus, nano-imaging forms an important part in the detection of newer pathways for neurodegeneration in PD.

Theranostics: GDNF can be delivered as a neuroprotective agent using microbubbles which are ultrasound responsive [68]. The use of such particles in simultaneous imaging and therapy can give rise to a theranostic approach in the management of Parkinson's disease. Similar therapies have been developed for the treatment of cancers [69] and is very promising. A similar approach is the development of magnetic-field responsive nanoparticles that are capable of crossing anatomical barriers like the BBB and respond to an external magnetic field by releasing the encapsulated therapeutic payloads [70]. The field of theranostics is promising and yet to be well explored in the treatment of Parkinson's disease.

Detection of cell loss: In 1999, Damier *et al.* described a protocol based on immunostaining a protein existing in afferent striatonigral fibers called calbindin D_{28K} [71]. Using this technique in post-mortem specimens, researchers could delineate 60% of all neurons containing dopamine present in the substantia nigra pars compacta inside the zone that has high calbindin (nigral matrix), and the remaining 40% was found to exist, packed together as the zones poor in calbindin (nigrosomes) in the form of invaginated pockets within the nigral matrix [71]. The group identified a mean reduction of more than half of the total dopamine-containing neurons in PD as compared to controls [71]. Besides, the substantia nigra pars compacta showed that the degree of loss of dopamine-containing neurons correlated with the duration of disease [71]. Thus, the pattern of cell loss in PD was found using this experimental tool. Further advancements in the imaging of cells using quantum dots and gold nanoparticles can advance this imaging modality to track PD progression.

Monitoring clinical abnormalities

One of the interesting applications in the diagnosis of PD is the detection of clinical abnormalities. Arrays of stretchable strain sensors based on silicon nanomembranes that conform well to the skin have been proposed as wearable devices for detection of clinical abnormalities [72]. Nanofabrication allows the manufacture of such sensors coupled with added features like presence of ultrathin serpentine interconnects to improve the precision of diagnosis [72]. Exposure to tension and compression on a human wrist are used for detection of frequency of tremors and can be of use in the continuous monitoring of PD.

Other interesting clinical abnormalities including change in handwriting have also been explored for the diagnosis of PD [73].

Nanotechnology platforms for drug delivery

Active targeting by Trojan horse approach

Nanotechnology based active targeting plays a crucial role in directing the neurotherapeutics at the target site through specific pathways. Nanoparticles are surface functionalized/modified with specific ligands like peptides (insulin, insulin like growth factor), antibodies or antigens for receptors/transporters that are expressed on BBB therefore, facilitating enhanced bioavailability through receptor mediated endocytosis into the brain. The peptidomimetic antibodies decorated on nanoparticles can be used as molecular Trojan horses to ferry large molecules like drugs and genes across the BBB [74,75]. Previous studies showed expression of different receptors mainly low-density lipoprotein (LDL) and low density lipoprotein related protein-1 and 2 (LRP-1,2) transferrin, P-gp efflux transporter on BBB [76]. The receptor mediated endocytosis involves ligand-receptor interaction resulting in endocytosis at the luminal side of BBB followed by translocation through the endothelial cytoplasm to the abluminal side and finally exocytosis allowing release of payload at the target site [77]. The payloads may be in the form of a drug or a gene. Gene therapy is becoming a popular approach to treat PD with some drawbacks in currently available therapies like poor permeability of the gene vector across the BBB after intravenous administration which limits the therapeutic efficacy. Local intracerebral administration of the gene vector at the target site is often limited by lack of patient compliance due to high invasiveness and repetitive injection schedule. Schlachetzki and colleagues discussed trans-vascular delivery, a novel approach for gene therapy to the brain using targeted nano-carriers [74]. This approach can be of two types- the more invasive one involves BBB disruption, using the intracarotid infusion of vasoactive compounds or hyperosmolar solutions and the less invasive one involves the targeting of certain endogenous transport systems within the BBB. As an example of the latter, Zhang *et al.* delivered tyrosine hydroxylase gene to the brain using a PEGylated immunoliposome system with a targeting moiety in the form of a murine monoclonal antibody (MAb) to the rat transferrin receptor [78]. Receptors like transferrin play an important role in transporting iron to the brain through receptor mediated endocytosis. The technology functions as a Trojan horse to ferry the gene across the BBB and demonstrated reversible normalization of tyrosine hydroxylase (TH) activity in the striatum along with favorable behavioral effects in experimental models of parkinsonism in rats [78]. Carroll and colleagues (2010) have reported the transferrin receptor antibody (OX26) conjugated PLGA nanoparticles loaded with antioxidant has significant potential against oxidative stress induced neurodegenerative diseases [79]. The *in vitro* study showed higher cellular uptake and decreased cell viability of RG2 rat glioma cells with targeted nanoparticles as compared to non-targeted nanoparticles through receptor mediated endocytosis. These findings suggest the potential of transferrin receptor in the treatment of other neurodegenerative diseases mediating the transport of drug across BBB [79].

Lactoferrin is a cationic iron binding glycoprotein from one of the transferrin receptor family. Previous studies on lactoferrin receptors reported that their expression on BBB facilitates the transport of lactoferrin to the brain [80]. Ji and colleagues in 2006 have demonstrated the greater brain uptake of lactotransferrin than that of transferrin and OX26 (an anti-transferrin receptor antibody) through endocytosis mediated by receptors in an *in vitro* BBB study. [81].Using this concept, Hu *et al.* in 2009 demonstrated the improved brain targeting potential of lactoferrin conjugated PLGA nanoparticles as

compared to unmodified nanoparticles [81]. Here lactoferrin acts as the ferrying moiety across the BBB to allow the Trojan horse- like delivery of payloads [81]. This finding showed higher brain uptake (3-fold) of lactoferrin modified nanoparticles in mice over non-modified nanoparticles [81]. Interestingly, in 2011, Hu *et al.*; further demonstrated the successful brain delivery of Urocortin (corticotrophin releasing hormone related peptide) loaded lactoferrin conjugated polyethylene glycol-polylactide-polyglycolide (PEG-PLGA) nanoparticles [82]. *In vivo* pharmacokinetic study of lactoferrin conjugated nanoparticles showed 2.49-times increase in AUC than that of non-conjugated nanoparticles in 24h. The *in vitro* cellular study on immortalized mouse brain endothelial cell line (b.End3) revealed that the uptake of conjugated nanoparticles occurred through clathrin mediated endocytosis with minimal adverse effect on cell viability [82]. Further the conjugated nanoparticles showed significant reduction of striatum lesions in 6-OHDA (6-Hydroxy dopamine) induced rat PD model. Together these findings revealed that lactoferrin is a promising targeting ligand to enhance targeting and accumulation of active to brain and lactoferrin decorated nanoparticles can help to deliver drugs more effectively in PD. Huang *et al;* (2010) demonstrated long term, non-invasive targeted gene therapy in rotenone induced chronic PD model using lactoferrin functionalized nanoparticles encapsulated with human glial cell line derived neurotrophic factor gene (hGDNF). The study revealed that intravenously administered lactoferrin decorated nanoparticles showed successful gene delivery to the brain through higher and long lasting GDNF expression in brain in experimental animals than that of single injection [83].

The LRP-1 and 2 receptors interact with various other molecules like apolipoprotein-E (apoE) in addition to lactoferrin and melanotransferrin which have been widely studied for targeting against PD. Wanger *et al.* in 2012 have reported the potential of low density lipoprotein receptor related protein (LRP-2) for specific and effective apolipoprotein mediated nanoparticulate transport and uptake by brain capillary endothelial cells [84].

A brief representation of the fate of actively targeted nanoparticles is provided in Figure 3.

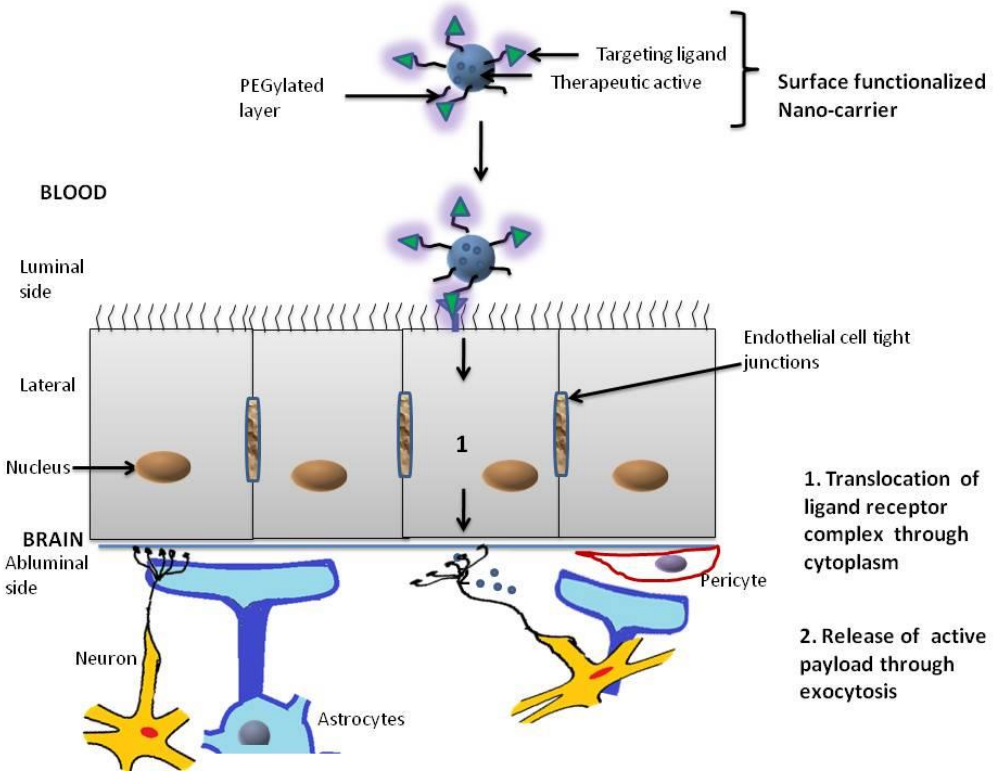

Figure 3. Use of targeted nanoparticles for active targeting in drug delivery for Parkinson's Disease

Routes of administration to enhance drug delivery across BBB

The presence of the BBB, limits the permeability of therapeutic agents to reach target sites in the CNS, resulting in insufficient bioavailability as well as poor treatment outcomes against neurodegenerative disorders. Several attempts have been made for effective and safe delivery of active into the CNS. Local drug delivery strategies include intrathecal, intraparenchymal and intracerebral injections directly at the target site for transporting the therapeutic active to the CNS. Direct drug delivery systems have potential to circumvent the BBB. Pillay *et al.*(2009) studied the binary cross-linked alginate scaffold embedded polymeric (cellulose acetate phthalate) nanoparticles loaded with dopamine which after intracranial implantation showed enhanced and sustained dopamine delivery within CSF in Sprague–Dawley rat model with reduced peripheral side effects as compared to orally administered L-DOPA [85]. Worly *et al*. (2008) developed acrylated 3'-sialyllactose (bioactive epitope recognized by various growth factor receptors like EGFR) embedded within poly[N-(2-hydroxypropyl methacrylamide)] (pHPMA) hydrogels for intracranial implantation [86]. On intracranial implantation in 6-OHDA PD model, the hydrogel served as a three dimensional substrate promoting neuronal tissue remodeling and angiogenesis with high affinity for receptors [86]. These finding demonstrated that polymeric nanoparticulate hydrogels can act as effective carriers for local delivery of drugs in PD. Nevertheless, these techniques are limited due to their invasive nature [77,87].

Recently, intranasal routes through olfactory or trigeminal pathways have been explored for transporting actives to the brain. Intranasal administration has the potential to circumvent the BBB via intracellular or extracellular pathways. The intracellular pathways include endocytosis (adsorptive or receptor mediated) into olfactory sensory neurons followed by intraneuronal transport (transcytosis) into trigeminal nerves whereas, extracellular pathways include paracellular diffusion to the lamina propria through perineural or perivascular channels [88,89]. Intranasal administrations offer benefits like rapid onset of action and reduced systemic toxicities for delivering the therapeutics to the brain. However, mucocilliary clearance limits the residence time when drug solutions are delivered intranasally. [90]. Recently, Wen *et al.* (2011) demonstrated that intranasal administration showed improved therapeutic efficacy of urocortin (corticotrophin releasing factor related peptide) when encapsulated in Odorranalectin (bioadhesive from lectin family) functionalized PEG-PLGA nanoparticles in hemiparkinsonian rats [91]. When administered intranasally, the Odorranalectin functionalized nanoparticles showed higher brain uptake and enhanced neuroprotection in experimental animals through direct pathway. Similarly, Migliore *et al.* (2014) reported the neurotropic and neuroprotective effect of GDNF following intranasal administration of GDNF loaded cationic liposomes (made up of dioleoylphosphatidylcholine (DOPC), cholesterol, and stearylamine) [92]. Intranasal GDNF protected the dopamine neurons within substantia nigra from acute 6-OHDP induced neuronal toxic insult [92]. Sharma *et al.* (2013) reported intranasal delivery of pluronic F-127 based thermoreversible gel containing levodopa encapsulated chitosan nanoparticles [93]. Chitosan being a muchoadhesive polymer plays an important role in improving drug absorption through nasal mucosa and can lead to opening of tight junctions across BBB. The *in vivo* findings revealed that intranasal administration showed maximum recovery of levodopa within the brain with chitosan nanoparticles [93]. Md and colleagues (2013) have studies the potential of intranasal route to enhance the brain delivery of bromocriptine (BRC) loaded chitosan nanoparticles in haloperidol induced PD mice [94]. When administered intranasally the BRC loaded nanoparticles showed pronounced reversion of catalepsy and akinesia induced by haloperidol. The biodistribution study revealed higher brain/blood ratio of BRC loaded nanoparticles following intranasal administration than that of intravenous route at 0.5 hours, indicating direct nose to brain transport of BRC circumventing the BBB. The gamma scintigraphy

study revealed higher brain uptake and enhanced antioxidant potential of BRC following intranasal administration [94]. A flowchart of the various routes and approaches to cross the BBB has been outlined in Figure 4.

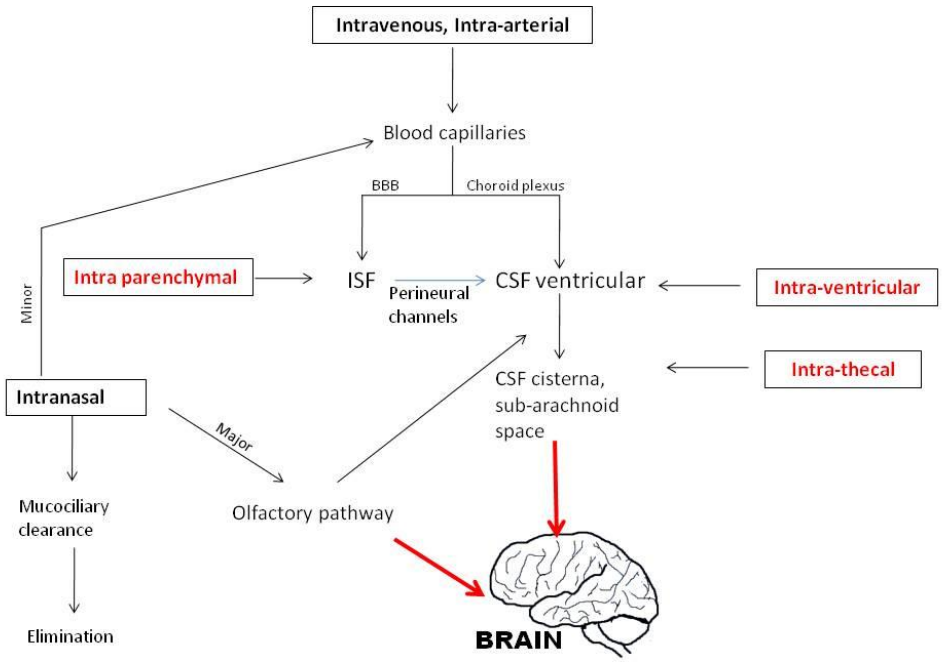

Figure 4. Novel Approaches for drug delivery across the BBB (The approaches are mentioned within boxes. Those in red are invasive approaches and those in black are relatively less invasive approaches for drug delivery.)

Passive Targeting (Macrophage loaded)

Nanotechnology platforms possess the ability to accumulate in a specific organ without active targeting moieties on its surface due to certain inherent characteristics. For example, in cancers, small size of nanoparticles allow them to preferentially accumulate at tumor sites by the Enhanced Permeation and Retention effect (EPR effect) which leverages the leakiness of blood vessels in a tumor and absence of lymphatics to deliver the nanoparticles [95]. In Parkinson's disease, the BBB is an important barrier that needs to be crossed for the delivery of therapeutics. This can be achieved passively through macrophages. In PD there is a chemokine-gradient that gets induced through neuroinflammatory responses which allow concentration of macrophages in the brain as outlined in Figure 5. Hence, it is possible to use macrophages as drug carriers for the delivery of various therapeutic payloads [96]. Batrakova *et al.* delivered catalase using bone-marrow-derived macrophage (BMM) systems to PD-affected brain regions in an animal model [97]. The self-assembled catalase/polymer complexes referred to as nanozymes that were 60-100 nm in size were taken up by the BMMs and showed sustained release for about 24 hours as compared to immediate degradation of the free enzyme [97]. This had implications in decomposition of microglial hydrogen peroxide that could reduce oxidative stress in PD. It was found that the nanozymes loaded into BMMs increased deposition of labeled enzyme in tissues *in vivo* in MPTP-treated mice as compared to free enzymes. The development of such nanoparticles loaded into macrophages can help in increased delivery

of therapeutics to the brain reducing the need and the costs associated with active targeting of nanoparticles.

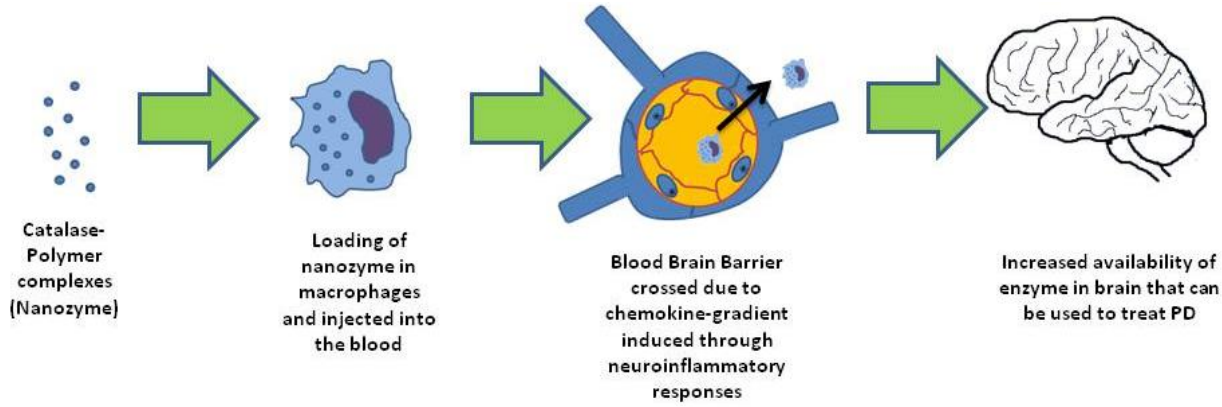

Figure 5. Use of Nanotechnology for passive targeting through macrophages for drug delivery in Parkinson's disease

Stimulus responsive nanoparticles

Nanoscale biomedical imaging for image guided therapy and diagnosis against neurodegenerative diseases is recently emerging. One of the widely studied multipurpose systems are magnetic nanoparticles (MNPs) with MRI contrast for applications like cellular and molecular imaging of various metastases, hyperthermia, drug delivery and tissue repair. In case of neurodegenerative diseases the presence of structural barrier likes BBB; restrict MNPs access to the CNS. Therefore smart engineering of MNPs with desired size and appropriate physicochemical properties is important to circumvent the BBB to gain access to the brain [70, 98]. Recently Qiao *et al.* (2012) developed target specific theranostics to facilitate the access of nanoparticles across BBB through endocytosis mediated by lactoferrin receptor. PEGylated magnetic nanoparticles (Fe_3O_4) surface modified with lactoferrin serve as a brain MRI guided delivery probe[99]. The *in vitro* porcine BBB model showed that amphiphilic PEG coating and lactoferrin conjugation facilitate the transport of MNPs across BBB [99]. The vascular imaging after intravenous injection of targeted MNPs confirm lactoferrin receptor mediated transcytosis with interaction between targeted nanoparticles and lactoferrin receptors expressed on microvascular endothelial cells [99]. Recently Hwang *et al.* (2009) reported ultrasound responsive stable perfluorocarbon nanobubbles (PNs) loaded with dopamine receptor agonist (apomorphine) for targeting the brain. The studies showed the improved stability and higher plasma residence time of apomorphine loaded PNs *in vivo* attributable to rigid cholesterol and phospholipid membrane. Ultrasound trigger altered the release profiles on demand. Erythrocyte hemolysis study demonstrated the safety profile (<10% hemolysis) of PNs [100]. Altogether these studies revealed the potential of stimulus sensitive nano-carriers for improving stability, administration frequency and on-demand release kinetics of therapeutics against PD. A brief representation of the use of nanobubbles as stimulus-responsive nanoparticles is provided in Figure 6.

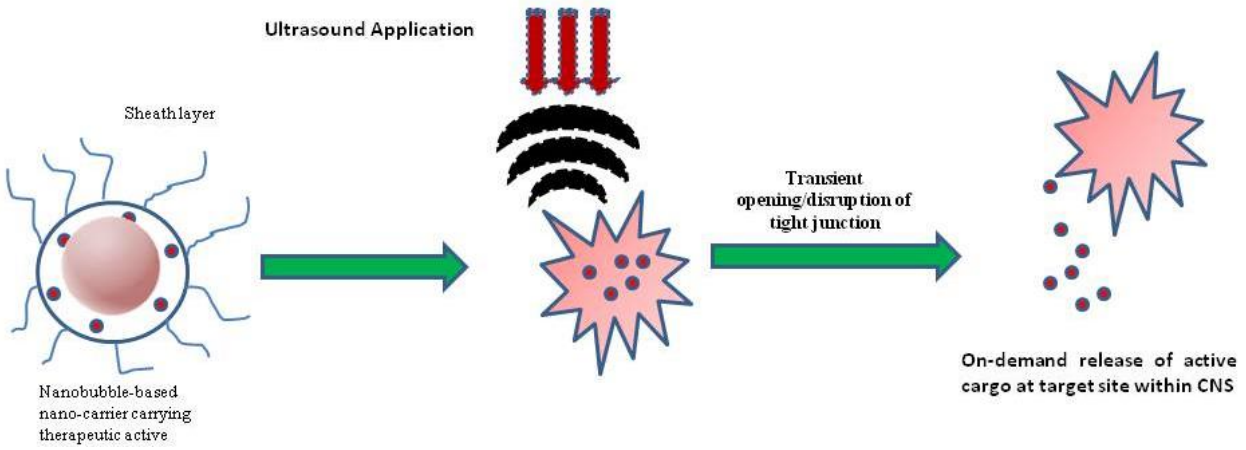

Figure 6. Use of Stimulus responsive nanoparticles for drug delivery in Parkinson 's disease with ultrasound (stimulus) responsive nanobubbles (nanoparticles) as an example

Neuroprotection

Neuroprotection is the ability for a treatment to prevent neuronal cell death by inhibition of the cascade resulting in cell dysfunction and eventually cell death. The relevance to PD lays in the fact that the pathogenesis of PD involves oxidative stress in substantia nigra through production of reactive oxygen species (ROS) and hydrogen peroxide by extensive oxidative metabolism of dopamine. Neurotrophic factors (like glial cell derived neurotrophic factor (GDNF)), fullerenes and nanoceria, show neuroprotection in PD [101,102]. The response from direct infusion of GDNF to the putamen had initial success but later could not demonstrate any significant benefits in controlled clinical trials due to development of antibodies and cerebellar degeneration [103]. This called for better modes of controlled delivery of GDNF. Wang *et al.* described the use of microbubbles loaded with GDNF which released GDNF on low-frequency ultrasound stimulation in the treatment of PD-induced in rats [68]. GDNF levels augmented in the striatum following low frequency ultrasound stimulation after the infusion of microbubbles and resulted in favorable biochemical and behavioral changes suggesting neuroprotective effects [68]. Thus, ultrasound responsive nanoparticles can be of use in the theranostics of PD- therapy by drug delivery and imaging due to the contrast provided by the microbubbles.

In another approach, a number of non-viral gene delivery vectors are being developed that have reduced toxicities and have comparable efficiencies in transferring GDNF genes into the target cells. As has been mentioned earlier, lactoferrin-conjugated nanoparticles have been used successfully for this purpose in PD rat models [104]. Similarly, Angiopep with a sequence of TFFYGGSRGKRNNFKTEEYC as the targeting ligand to mediate BBB transport and cellular internalization was conjugated to poly-L-lysine-based dendrimer nanoparticles (dendrigraft poly-L-lysine) via hydrophilic polyethylene glycol (PEG) [105]. These nanoparticles constructs allowed effective gene expression both *in vitro* and in rat models of PD [105]. In addition to the delivery of neurotrophic factors, the use of fullerenes and nanoceria is of great interest. Pretreatment with polyhydroxylated fullerene derivative C(60)(OH)(24) at ≥20 μM concentrations were used by Cai *et al.* for the purposes of neuroprotection [106]. In an *in vitro* cellular model of Parkinson's disease, significant protective effects as reflected by cell viability, mitochondrial function; levels of reactive oxygen species decreased and DNA and proteins damage were reduced with polyhydroxylated fullerenes [106]. Also, fullerenes in the form of carboxyfullerenes (C3) showed neuroprotection in *Macaque fascicularis* monkeys [107]. The technology leveraging the antioxidant properties of fullerenes in the treatment of Parkinson's disease is being commercialized and the leading companies involved are The

Bronx Project, Inc. (TBP), Tego BioSciences Corporation (Tego) and Luna Innovations Incorporated (Luna) [108]. In addition to this, cerium oxide (CeO_2) is being used for its capability of neutralizing and reducing harmful reactive oxygen species in a redox reaction [102]. Due to the oxygen vacancies on the CeO_2 surface and presence of both trivalent and tetravalent states of cerium ions, during the redox reaction the ions can flip-flop between the two oxidation states [102]. Leveraging this scavenging role of cerium oxide, Pinna *et al.* showed a protective role of nanoceria both *in vitro* on PC12 cells survival and dopamine metabolism in manganese-induced Parkinson's Disease [102].

Other approaches in management of Parkinson's Disease based on nanotechnology

Neural prosthetic electrodes for Deep brain stimulation

Bilateral deep brain stimulation (DBS) of the subthalamic nucleus (STN) through electrical stimulation using implanted neural prosthetic electrodes is a proven and FDA-approved treatment modality for advanced Parkinson's disease [109]. Deep brain stimulation has been found to be better than medical therapy in producing improvements in motor symptoms [109,110]. However, the precision of DBS is an important issue that needs to be tweaked to address the ethical implications of altering brain circuits with this technique [111]. For this purpose, neural stimulation has employed a wide range of metals and metal alloys as materials for fabrication of electrodes in neural stimulation. These include noble metals (Platinum), titanium oxide, activated iridium, tantalum oxide, and silicon [112]. However, these materials include limitations in the form of low charge injection limit allowing only large electrodes with low current density to be used [113], Faradaic reactions (consisting of electron transfer between electrode and electrolyte resulting in either reduction or oxidation of a particular chemical species in a given electrolyte) leading to diffusion of toxic products or the corrosion of electrodes [114] as well as issues with biocompatibility with the electrode materials [114].

Carbon Nanotubes- One of the most versatile nanotechnology options explored for neural prosthetic electrodes are carbon nanotubes (CNTs) which have the unique combination of being strong (Young's modulus more than 1 TPa) as well as flexible. Both of these are extremely important for fabrication of penetrating electrodes in neural prostheses [114]. Besides, to serve as an electrode for neural stimulation it should be able to deal with the critical trade-off between high charge injection limit and absence of Faradaic reactions. Wang *et al.* used multiwalled carbon nanotube (CNT) pillars to fabricate microelectrodes [112] and successfully created a neural interface prototype device using CNT microarrays containing CNT microelectrodes. The neurons could grow and differentiate on the interface as well as could be repeatedly excited. The important parameters that were identified included the high charge injection limit offered by these functionalized hydrophilic CNT microelectrodes without faradic reactions [114]. This ensured the efficient and safe electrical stimulation of neurons and had several uses in diagnosis and therapy of neurological diseases like PD [114].

Conducting Polymers- Another group of innovations in this field is the development of coatings on neural prosthetic implants using conductive polymers containing alternating double and single bonds in their structure [115]. Polypyrrole (PPy), poly(ethylene dioxythiophene) (PEDOT), polyaniline and polyterthiophene are common examples of such polymers [115]. George *et al.* found polypyrrole implants used in microfabricated neural prosthetics allowed the growth of neural connections on all surfaces [116]. However, these implants showed tissue reactions in the brain in the form of gliosis [116] which was one of the major causes of failure of these electrode arrays within a few weeks after implantation [117]. To tackle this problem, Wadhwa *et al.* proposed PPy based coatings on the electrodes

with electrically controlled local delivery of dexamethasone (Dex), a drug which is known to reduce tissue reactions and gliosis [117]. Micromachining and one step electropolymerization was used for the deposition of a thin film (several nm) of PPy and the drug on the gold coated electrode surface for the fabrication of this smart electrode capable of responsiveness to electrical stimulus [117]. The added advantage of use of polypyrrole in such smart platforms is the fact that redox reactions cause the charging and discharging of these polymers and can lead to the movement of hydrated ions, drugs and growth factors in and out of the bulk of the polymer [117]. The drug loaded into the electrode was released in the buffer solution in the presence of an electrical trigger that was applied through cyclic voltammetry [117].

Conductive polymer hydrogels- The increased biocompatibility of these drug-eluting coatings on smart microelectrodes led to research towards increasing the drug loading and stimulus responsiveness of the electrodes. This lead to conducting polymers being incorporated inside hydrogel-support matrices, a class of materials called conductive polymer hydrogels (CPH) [118]. Such a matrix can allow more drug incorporation along with faster release [117]. These properties can be further enhanced through the increase in surface area by creating nano-fibrous morphology employing nano-templating techniques [117]. Such devices can serve as mechanical buffers in the interface between the hard electrodes and the soft brain tissue improving contact with viable neurons while increasing biocompatibility and limiting protein adsorption to the neural prosthetic electrodes [117,118]. According to a study by Nguyen *et al.,* the incorporation of a mechanically-compliant implant in the brain parenchyma can reduce the inflammatory response in comparison to stiffer systems [119]. Thus, CPH coated electrodes are biocompatible by themselves and can be additionally used to deliver drugs and growth factors locally. . Kim *et al.* used PPy grown electrochemically on the scaffolds made of hydrogels followed by deposition on the surface of neural prosthetic devices [120]. The coating was found to reduce impedance and improve electrical properties of the electrodes [120]. However, hydrogels have the inherent property of a high swelling ratio and its swelling *in situ* can lead to increase in the distance from the brain surface and the microelectrode reducing the efficiency of deep brain stimulation. To deal with this problem, bio-inspired hybrid microelectrodes were fabricated by De Faveri *et al.* in which they used highly biocompatible fibrin coatings [121]. The fibrin coatings reduced host reaction within 7 days of implantation and allowed growth of both neurons and astrocytes [121]. But most importantly, the fibrin coating displayed a smaller swelling than other types of hydrogels and allowed the control of coating thickness [121]. Thus, the problem of hydrogel swelling could be tackled with suitable bio-inspired coatings.

Thus, nanotechnology is being increasingly employed for the development of better neural prosthetic electrodes for deep brain stimulation. Further advances in the fabrication and stimulus responsiveness of these technologies can help incorporate DBS as a safer treatment modality in the management of Parkinson's disease.

Nanotechnology in neurosurgery

After the introduction of levodopa half a century ago, neurosurgery was abandoned for the management of Parkinson's disease. However, the past decade saw the resurgence of the surgical treatment of PD using pallidotomy and thalamotomy [103]. This was due to the inherent drawbacks of drug therapy in which the prolonged administration of drugs lead to drug-induced motor symptoms [103]. With the advent of the age of nanotechnology, there have been great strides in the precision in neurosurgery.

Laser Axotomy- One of the most intriguing techniques in the field of nanotechnology is the use of

femtosecond laser axotomy which is being developed as a neurosurgical tool with 100% efficiency, precision in the range of nanometers and high speed. The most widely cited example of this precise technique is by Yanik *et al.* (2011) where femtosecond laser axotomy was used to cut single axons inside the roundworm *C. elegans* by using focused nano-scale near-infrared laser pulse energies (10–40 nanojoules) of 200-femtoseconds [122]. This resulted in the vaporization of femtolitres of axon volumes and subsequent cutting of the axon processes [122]. Besides, the technique was robust allowing the use of confocal fluorescence imaging and laser scanning brightfield for real-time imaging from the first incision to the end [122].The surgical technique has improved understanding of a number of processes in nerve cell injury [123] and has also paved way for an all new concept of nanomanipulation.

Vibrating micropipette- Another widely popular technology is the use of a rapidly vibrating (100 Hz) micropipette (tip diameter in nanometers) which when dragged across the dendrite of a selected neuron can help in dissection of apical dendrites [124]. This provided a simple system for cutting dendrites from single neurons without damaging the cell [124].

Nanotweezers- The word nanotweezers is being widely used with different meanings but with the same purpose of precise manipulation in the nanometer range. The nanotweezers may be of nanoscale electromechanical systems based on carbon nanotubes fabricated on pulled glass micropipettes as described by Kim *et al.* [120]. Voltages that are applied to these electrodes could open or close the free ends of the nanotubes allowing grabbing and manipulation of submicron clusters and nanowires [120]. The mechanical capabilities of such nanotweezers can be put to use for grabbing and manipulating neurons as well. A rather higher level of precision in manipulation is by the use photoswitchable crosslinkers which in response to light can manipulate the normal physiological signal or induce structural changes in peptides and ion channels allowing molecular level manipulation of cells [125]. The use of light is being increasingly used for the study of disease circuitry in Parkinson's disease especially through optogenetics which is a novel technique of gene delivery through encoding of proteins capable of instilling light sensitivity to neurons [126]. These technologies once translated can improve the precision of neurosurgery to molecular ranges.

Nanotechnology and the use of smart nanoparticles can greatly improve the precision of such surgical devices from cutting neuronal processes to modifying ion channels using light. As these devices are gradually becoming popular in the lab, their translation to bedside is possible in the near future. The essential precision that these nanotechnological tools add, can lead to a safer neurosurgical option for patients with PD as with many other neurological diseases.

Tissue Engineering Approaches - Finding the ideal platform

The dopaminergic neurons in the substantia nigra responsible for cortical and thalamic regulation are lost in PD leading to motor dysfunction (tremor, rigidity, and bradykinesia) and non-motor symptoms (depression, anxiety) [103]. The transplantation of fetal tissue in the form of human fetal ventral mesencephalon was one of the most promising treatments in the 1980s and stimulated a number of clinical trials with varied protocols and endpoints [127,128]. There were reports of clinical improvement and two decades later, after sufficient evidence of safety and efficacy of these treatments, the National Institutes of Health, USA started two clinical trials in the early 2000s [127]. The trials found the therapy to be of little benefit to patients and caused some of the patients to become worse with symptoms like graft-induced dyskinesias (GID) [127,129]. However, a decade later, the debate is still on regarding the efficiency of stem-cell grafting [128,129]. Besides the concerns in the clinical-trial design and ethical issues, the

technology needed to be safe [127,128]. These concerns are more pronounced with embryonic stem cells (ESCs) because of their ability to differentiate into a number of cell types [127]. A potential solution is the use of patient derived cells and then induced pluripotent stem cells (iPSCs) [127]. However, the concern still lies in the fact that the patient's own cells may be susceptible to the PD pathology [127]. Thus, it is the trade-off between optimal ability to proliferate while dodging the pathogenesis of PD that forms the basis of development of a safe treatment for PD. Direct infusion of growth factors like glial cell–derived neurotrophic factor (GDNF) to the putamen in patients with PD has been found to be favorable and are being seen as prospective agents for neuroprotection [103]. However, these approaches are still to be verified as clinical trials reflect conflicting evidence [103]. Tissue engineering tries to address these issues using stem cells and growth factors in suitable scaffolds for the controlled regeneration of various tissues. It is being increasingly used for neural stem cell engineering for Parkinson's disease.

Nanoparticles for delivery of versatile payloads: One of the approaches in tissue engineering for PD is the use of small molecules as triggers to allow the differentiation of stem cells into the neurons. Santos *et al.* used retinoic acid-loaded polymeric nanoparticles to induce such differentiation in subventricular zone neural stem cells [130]. The nanoparticles leveraged the electrostatic interaction of polyethylenimine (polycation) with retinoic acid and dextran sulfate (polyanion) [130]. The intracellular delivery of retinoic acid using nanoparticles increased the differentiation of stem cells into neurons as was confirmed using gene expression profiles. Such technologies can be of great use if nanoparticles containing retinoic acid are manufactured for targeting stem cell sequences to control in time and space the differentiation of stem cells [130].

Scaffold design: In addition to using small molecules for the differentiation into a particular lineage, there can be changes made in the substrate too allowing nanoknitting of neurons on scaffolds [131]. Ni *et al.* developed a Self-Assembling Peptide Nanofiber Scaffold (SAPNS) 6–10 nm in diameter to enhance differentiation of ESCs as well as iPSCs into dopaminergic neurons in a 3-dimensional culture [132]. This was confirmed using gene expression, maturation, immunocytochemistry and dopamine release all of which proved genetically and functionally differentiated dopaminergic neurons in the scaffold [132]. Hence, these materials have the potential to be used for neuroprostheses. In addition to this, materials used in neural prosthetic electrodes have also been used in tissue engineering. Conductive polymer hydrogels (CPH) have the advantage of allowing cell immobilization while increasing the surface area and electrical properties necessary for nerve growth and regeneration [117,118,120].

Thus, nanotechnology-based platforms are increasingly being used to tackle the challenges of stem-cell delivery. The present day platforms based on nanotechnology would allow a more precise and accurate delivery of stem cells for the management of Parkinson's disease. A brief summary of the use of nanotechnology in these therapies is represented in Figure 7.

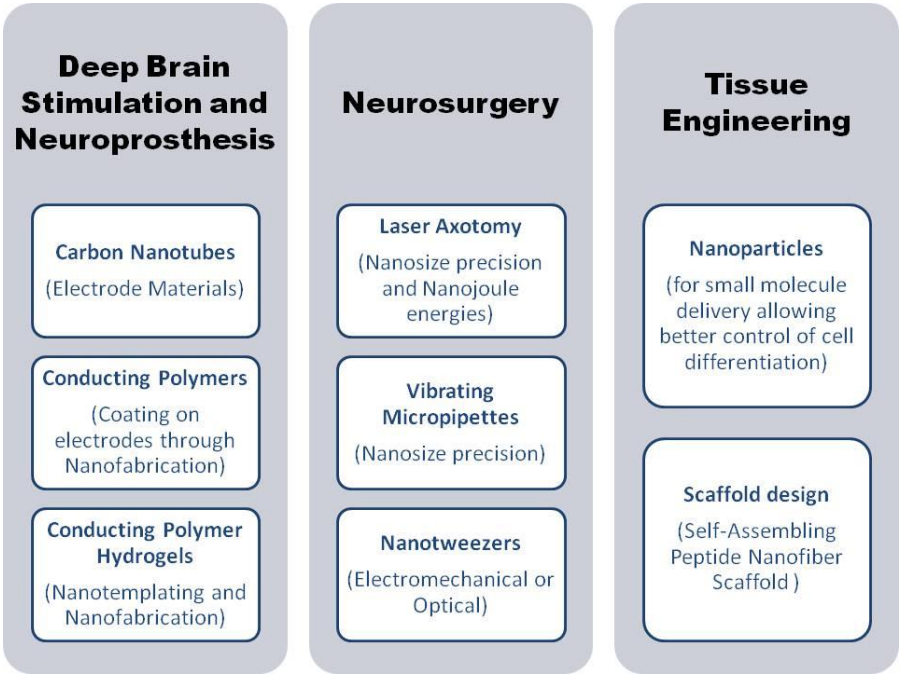

Figure 7. Nanotechnology-based platforms for the management of Parkinson's disease

Translation, Future Prospects and Concerns

The increased awareness of PD and the accelerated research towards its management has lead to the infusion of a number of technologies into the scenario. Nanotechnology is a novel and versatile addition to the fight against PD. There have been efforts in accelerating research in the identification of novel biomarkers and strategies for the cure for PD. This has led to a lot of research reach from labs to the bedside. Nanotechnology has the ability to develop smarter sensors for the diagnosis of PD using these novel biomarkers and imaging modalities. It is also being used for the targeted treatment of PD, in neuroprotection and in stem cell therapies.

Multinational technology companies are also entering the arena of nanotechnology to treat Parkinson's disease. Google Inc. has recently filed a patent for a wearable device in the form of a watch or bracelet that can direct functionalized nanoparticles (like magnetic nanoparticles through the magnetic field generated by the device) into the subsurface vasculature present close to the device [133]. The patent claims that there is a potential for the use of such devices in the treatment of PD by targeting certain proteins [133]. Besides, the clinical translation of research has lead to the development of a number of commercial entities. The Bronx Project, Inc. (TBP), Tego BioSciences Corporation (Tego) and Luna Innovations Incorporated (Luna) [108] are involved in the development of fullerenes for neuroprotection in PD. Parkinson's Pen (by Manus Neurodynamica Ltd.) is another patented technology that uses the changes in handwriting in combination with a sensor device on the pen to analyze the force exerted by each of the fingers, and the changes in handwriting [73]. Such pressure sensors and detectors can be of use to detect PD. Parkinson's Pen itself in its initial testing was able to distinguish 10 people with PD from age- and sex-matched controls with 90% sensitivity and 80 % specificity [134]. A clinical trial with a larger sample size is underway to confirm the sensitivity and specificity of the promising device [134]. This technology will have applications in diagnostics, pre-symptomatic screening, disease monitoring, treatment response and rehabilitation. The company is currently conducting clinical trials to this end. Thus, there is a lot of translation that is taking place from diagnosis to rehabilitation of PD patients.

Another area that is developing a lot in PD is the use of stem cells. Nanotechnology affords a targeted and controlled delivery and differentiation of stem cells for the therapy of PD. However, one of the biggest concerns in the present day is the development of treatments of PD using unproven cell-based treatments which pose serious threats to research translation [128]. These therapies pose increased risk of tumor formation [126]. Such therapies continue to be performed without intensive testing *in vitro* and *in vivo* and pose a serious risk to jeopardize the spark of hope that is gradually infused in the sphere due to the advent of tissue engineering and nanotechnology [128].

In addition to this, there are ethical concerns raised regarding neurology and nanotechnology and their potential impact for which there is a need for a multidisciplinary bioethical enquiry [135]. Also, there are conflicting reports regarding toxicity of certain nanoparticles which cross the blood brain barrier [136-138]. This has called for further evaluation of nanoparticles for surface properties, size and constituents for finetuning the therapeutic effects while avoiding toxic effects [136]. For drug delivery formulations, it is imperative to increase the therapeutic window which is the range of dosage that can effectively treat disease while staying in the safety range and without inducing adverse effects [136]. Comprehensive testing for efficacy and toxicity must be performed before the introduction of nanotechnology-based systems into the market [136]. According to the Royal Society/Royal Academy of Engineering report (2004), carbon nanotubes should be comprehensively tested as “new substances” for their potential toxicities under UK and European Health, Safety and Environment legislation before they are introduced in the market [57]. However, each nanoparticle is unique and it is necessary to understand the specific issues associated with each type of nanoparticle for evaluation of toxicity issues. Use of green chemistry, degradable materials, and regulatory approvals for nanotechnology-based products are the positive signals towards an optimistic future with nanotechnology, the effects of which can be of potential benefit to patients with PD.

Conclusions

Parkinson’s disease has a huge impact on the lives of the patients with tremendous socioeconomic effects. Nanotechnology can serve as a viable alternative to the present day diagnosis and treatment of PD by providing novel, affordable and accessible devices. Nanotechnological platforms are being increasingly developed with applications in the management of PD. The optimism in the translation of these technologies from labs to bedside can be seen with increasing interest by grant agencies, companies and physicians towards their development. The tremendous potential to tweak nanoparticles in the nanoscale can prove to be of great help in the fight against Parkinson’s disease.

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