Growing synergy of nanodiamonds in neurodegenerative interventions

Jackson Saraf[1], Kiran Kalia[1], Pallab Bhattacharya[1] and Rakesh Kumar Tekade[2]

[1] Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER) Ahmedabad, Gandhinagar 382355, Gujarat, India
[2] Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER) Ahmedabad, Gandhinagar 382355, Gujarat, India

Neurodegenerative diseases are complex in both their nature and prognosis. The difficulties associated with penetrating the blood–brain barrier (BBB), achieving site-specific targeting to the brain, and identifying the genetic etiologies responsible make treating neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and stroke, challenging. The aim to treat disease at the molecular level has galvanized nanotechnology research. Among the forms of nanoparticles (NPs) explored thus far, nanodiamonds (NDs) have shown great potential. Their unique physicochemical properties, such as a nanometer size range, stable and inert core, tunable surface, intrinsic fluorescence without photobleaching, negligible toxicity, and the ability to form complexes with drugs, highlight their theranostic potential. The ability of NDs to penetrate the BBB and target specific affected areas of the brain could take research one step closer to understanding the underlying disease etiology and unlocking more efficient methods of delivering neuromedicine to specific areas of the brain. Here, we explore interactions between NDs and the neuronal circuitry with a focus on the therapeutic potential of NDs as treatments for neurodegenerative diseases.

Introduction

Neurodegeneration is a significant global health threat. It is estimated that number of people aged 60 years or older will eclipse 2 billion over the next 35 years and this expanding population is accompanied by increases in age-related neurological diseases [1]. Neurodegenerative diseases, which fall under noncommunicable diseases as classified by the WHO, are responsible for 54% of the global disease burden when disability-adjusted life years (DALYs) are taken into account [2]. DALYs are the sum of years of life lost (YLLs) and years lived with disability (YLD), the formula obtained through Bayesian meta-analysis [3]. The term 'neurodegenerative' is generally used to denote any pathological condition affecting the neurons [4]. Factors responsible for neurodegeneration can be either genetic or environmental sources, or a combination thereof [5]. In most cases, age is the main determining factor; however, what makes neurodegenerative diseases so difficult to assess and diagnose is the involvement of other pathologies, including inflammation, oxidative stress, endocrine dysfunction, hypertension, diabetes, ischemic insults, smoking, head injury, trauma, infections, immune and metabolic dysfunction, tumors, chemical exposure, and vitamin and nutritional deficits [6]. Overall, 'neurodegenerative' serves as an umbrella term and is a significant component of diseases that might not involve neuronal degeneration per se (e.g., epilepsy or schizophrenia) but that are characterized by varying degrees of cognitive and motor dysfunction [7].

The development of prophylactic therapies for neurodegenerative diseases is hindered by the lack of convincing serological biomarkers of most of these diseases. If such markers do exist, they can involve invasive techniques that are not welcomed by

Corresponding authors: Bhattacharya, P. (pallab.bhattacharya@niperahm.ac.in, Pallab.bhu@gmail.com), Tekade, R.K. (rakeshtekade@gmail.com, rakeshtekade@niperahm.ac.in)

https://doi.org/10.1016/j.drudis.2018.10.012

patients [8]. Most diagnoses occur via magnetic resonance imaging (MRI) of the brain [9].

Neurodegenerative diseases not only affect patients, but also their friends and relatives. Along with deteriorating physical and mental health, financial constraints also become a burden. This cascade of physical, emotional, economic, and societal factors all exacerbate the quality of life of those with these chronic illnesses [10]. What is often observed is that these psychosocial factors, including depression, might derail the quality of life of the patient more than the disease itself [11]. Despite rapid advancements in medicine and biotechnology, no therapies have yet been developed to stop neurodegeneration [12]. Most treatments focus on treating the symptoms, although targeting the underlying cause of most neurodegenerative diseases remains the main focus for research. In addition, many chronic neuronal disorders progress without manifesting any symptoms for early diagnosis and prognosis.

The barrier to effective treatment

The BBB is a daunting hurdle when treating neurodegenerative disease: its role to protect the sensitive neuronal system of the brain also prevents treating the same when it has become infected or dysfunctional [13]. The BBB comprises endothelial cells that differ from those supplying blood to the rest of the body. These cerebral endothelial cells are bound by astrocytes, contain a surplus of tight junctions, lack fenestrae and transendothelial channels, and also have fewer pinocytic vesicles; they are known as the brain microvascular endothelial cells (BMECs) [14]. The BBB also rejects low-molecular-weight (<500 kDa) and high lipophilicity molecules that would normal pass through the barrier. This occurs through efflux systems, including P-glycoproteins, multidrug resistance proteins, breast cancer resistance proteins, and multiorganic anionic transporters. To bolster this defensive front, an enzymatic barrier inactivates any active central nervous system (CNS) agents and degrades them before they reach the desired target sites [15]. Such a complex barrier is one of the main reasons why there is a dearth of effective CNS active moieties on the market. The Comprehensive Medicinal Chemistry (CMC) database reports that only 5% of the 7000 enlisted drugs are CNS active, and mostly focus on insomnia, schizophrenia, and depression [16]. Although different techniques to increase CNS bioavailability have been used, they only marginally increase the total amount of drug reaching the CNS. Furthermore, they also lead to more adverse effects. Most importantly, concurrent neuromedicine aims to bring symptomatic relief by alleviating the outcomes of the disease rather than the disease itself [17]. Current research focuses on developing new methods and formulations that increase the likelihood of targeting the underlying pathology of neurodegenerative diseases [18].

Getting smaller: nanoparticles

A focus of NP technology research is to try to target the pathology of the disease at the molecular level. To do so, drugs must be delivered in a particle size range capable of easily penetrating most cell membranes to interact with the targets responsible [19]. This approach makes treatment efficient with fewer adverse effects because there is less interaction with the surrounding cellular components. Ideal NPs must have characteristics including a tunable surface, enhanced solubility, site specificity and multifunctionality, inability to interact with the biological system (non-immunogenic) or confer toxicity of their own, prolonged duration in blood (long half-life), control of drug release, increase drug bioavailability, and, most importantly, be of a nanoscale size capable of penetrating the various cellular membranes to elicit the desired therapeutic effect [20]. What also makes NPs so enticing is their flexibility and versatile nature; they not only expedite the delivery of drugs, but also offer bioimaging via fluorescent properties and MRI compatibility [21,22], act as probes for DNA, RNA, and nucleic acids [23], aid in the detection and identification of various biomarkers [24], can be used to induce hyperthermia to eradicate tumors [25], and can even be used in phagokinetic studies [26]. A range of NPs have been formulated, including solid lipid NPs, liposomes, dendrimers, nanogels, polymeric NPs, metallic NPs (gold, platinum, and iron oxide), and diamondoids (carbon nanotubes) [27].

Despite this development, only a few of these formulations have shown the ability to cross the BBB. Size is the most important property for crossing the BBB, although shape also has a role. Most studies have been conducted on spherical NPs, but nanorods have shown even higher adhesion toward BMECs [28]. Zeta potential also has an important role, with low–high negative zeta potential drugs being more able than higher positive zeta potential drugs (>15 mV) to immediately disrupt the BBB [29]. Surface modification has helped overcome some of the initial shortcomings of NPs, such as their poor solubility and drug loading. Newer techniques involve the conjugation of various ligands to NPs. Strategies have been used to tackle the problem of crossing the BBB, such as ligands that directly communicate with receptors of the BBB [30]; ligands that can bind proteins from the bloodstream and exploit them with receptors located on the BBB (the Trojan horse effect) [31]; ligands that enhance the lipophilicity and negative zeta potential [32]; and ligands that prolong the half-life in the systemic circulation [33].

Overcoming cytotoxicity

The cytotoxicity of NPs is another issue to consider when utilizing them for biomedical applications and bioimaging. For instance, Zhang *et al*. observed the neurotoxic effects of titanium dioxide NPs when given intranasally in mice [34]. Chen *et al*. observed the liver and lung toxicity of zinc oxide when administered intravenously but not orally [35]. Cationic dendrimers show a high cytotoxicity profile and require surface modifications [36]. Polypropyleneimine dendrimers and higher generation dendrimers compromise cell membrane integrity [37]. Significant surface modifications are needed to reduce the cytotoxicity observed with dendrimers and carbon nanotubes [38]. Poly-(D,L-lactide co-glycolide) NPs are affected by low efficiency resulting from uptake by the reticuloendothelial system [39]. Hyaluronic acid-grafted poly-(D,L-lactic)-co-(glycolic acid) (PGLA) NPs displayed greater cell death than when using doxorubicin alone [40]. In other cases, even carbon allotropes, such as carbon nanotubes and carbon dots, showed cytotoxicity when given *in vivo* [41]. Carbon NPs, such as carbon nanotubes, C60 fullerenes, and quantum dots, and transition metals, such as silver, can result in oxidative stress and cytotoxicity [42]. Compared with other nanomaterials and carbon allotropes, such as

carbon nanotubes, fullerenes, carbon dots, and carbon nanospheres, NDs showed the least amount of toxicity. Yu *et al.* reported low cytotoxicity via an MTT assay in a human kidney cells (293T line) when exposed to NDs [43]. Paget *et al.* extensively studied the cytotoxic and genotoxic profiles of carboxylated NDs *in vitro* using human liver, intestine, lung, and kidney cell lines. Using sensitive techniques, such as cell impedance, cell flow cytometry, and γ-H2Ax foci detection (most sensitive for detecting double-strand breaks in DNA), the authors observed negligible cytotoxicity and genotoxicity in all cell lines at ND sizes of 20 nm as well as doses ranging from 10 to 250 mg/ml [44]. In another study, four different types of detonated ND were studied for cytotoxicity after endocytic uptake by normal and cancer urothelial cell lines over 24, 48, and 72 h, and showed high viability with ND sizes ranging from 100 to 700 nm and doses of 5.5, 11, and 22 mg/ml [45]. The study highlights detonated NDs as a drug delivery system (DDS) for urological therapy. In another key report, NDs were studied for their interaction with erythrocytes and were found to be hemocompatible [46].

Enter nanodiamonds

NDs are exciting carbon-based NPs with a unique set of physical properties. They contain a truncated octahedral structure with 14 faces, 36 edges, and 24 vertices. This structure was elucidated using high-resolution transmission electron microscopy and X-ray diffraction [47] (Fig. 1 [48]). However, their geometry varies widely (because of differing synthesis and purification techniques) and a lack of extensive studies has resulted in the assumption that NDs are spherical [49]. However, a recent study by Ong *et al.* suggested NDs to be more flake-like in structure, with rougher edges than previously hypothesized [50]. Their structure results from graphitic (sp^2) and diamondoid (sp^3) bonds. These bonds are interchangeable and endow flexibility to the geometric orientation, particularly around the curved surfaces [51].

More importantly, shape has an interesting role in ND internalization by cell membranes. Although it remains to be elucidated exactly how NDs are able to cross cell membranes, especially the BBB, the shape of NDs could help determine the methods used for cellular internalization, particularly via the BBB. It has been hypothesized that, compared with their rougher-edged counterparts, rounder-edge NDs induce greater internalization by initiating membrane deformation, possibly as a result of their intrinsic rounder surface, enabling them to be in greater contact and interact with the membrane interface and requiring less energy to stimulate endocytosis [52]. In addition to receptor-mediated endocytosis and pinocytosis, this could be another underlying mechanism by which NDs cross cellular membranes, including the BBB [53–55] (Fig. 2).

Such an intricate structure can result in a vast and versatile set of features. NDs come in a range of sizes (1–150 nm) and can be classified as nanocrystalline particles (5–150 nm or more), ultrananocrystalline particles (4–5 nm), and diamondoids (1–2 nm) [56]. ND synthesis techniques vary from detonation (most commercially used) [57], laser ablation [58], high-energy ball milling of high-pressure high-temperature (HPHT) diamond microcrystals [59], carbide chlorination [60], plasma-assisted chemical vapor deposition (expensive and not feasible for large-scale production), ultrasound cavitation, and graphite irradiation [61]. However, size is not the only thing that differs among the different synthesis techniques; the shape, structure, surface aspect ratio, surface chemistry, and functionalization can also vary, creating complexities in understanding ND aggregation behavior and interactions

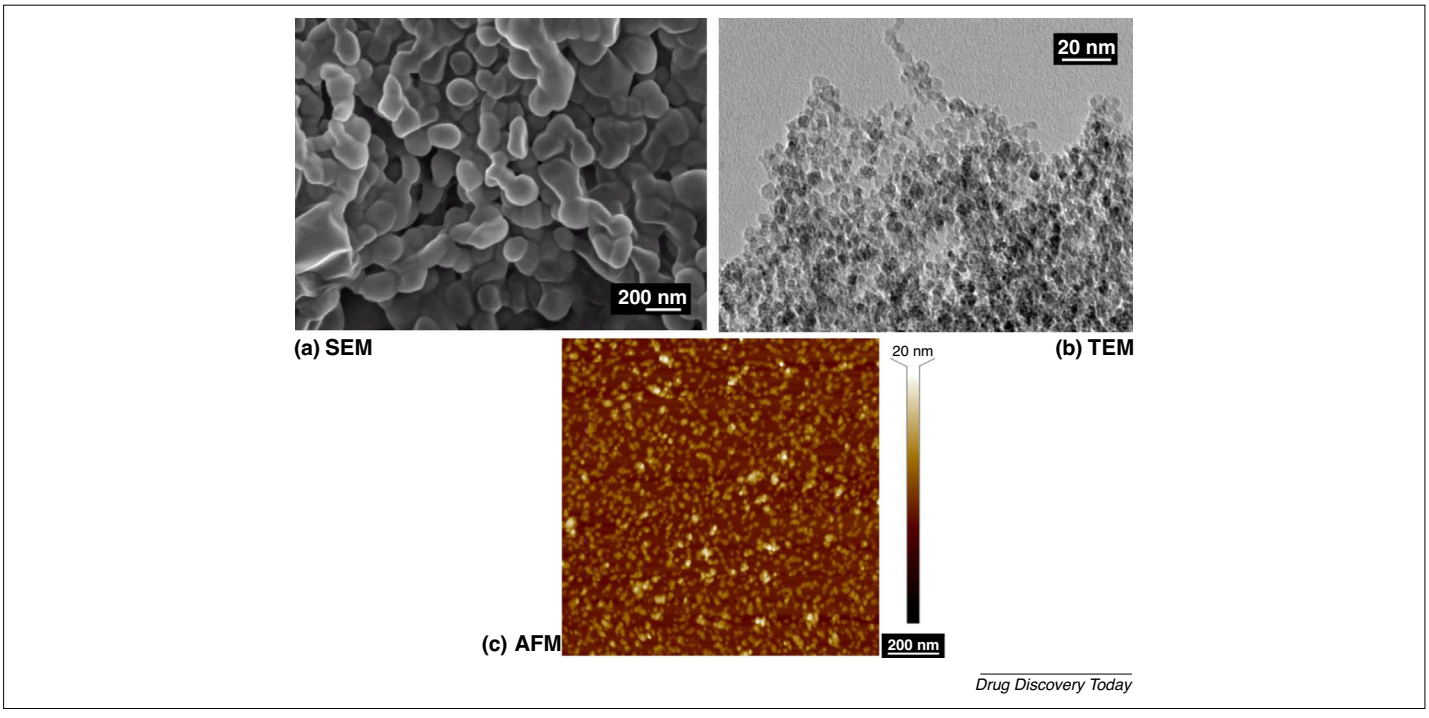

FIGURE 1

Observation of nanodiamonds (NDs) by using (a) scanning electron microscopy (SEM); (b) transmission electron microscopy (TEM); and (c) atomic force microscopy (AFM). Reproduced from Ref. [48].

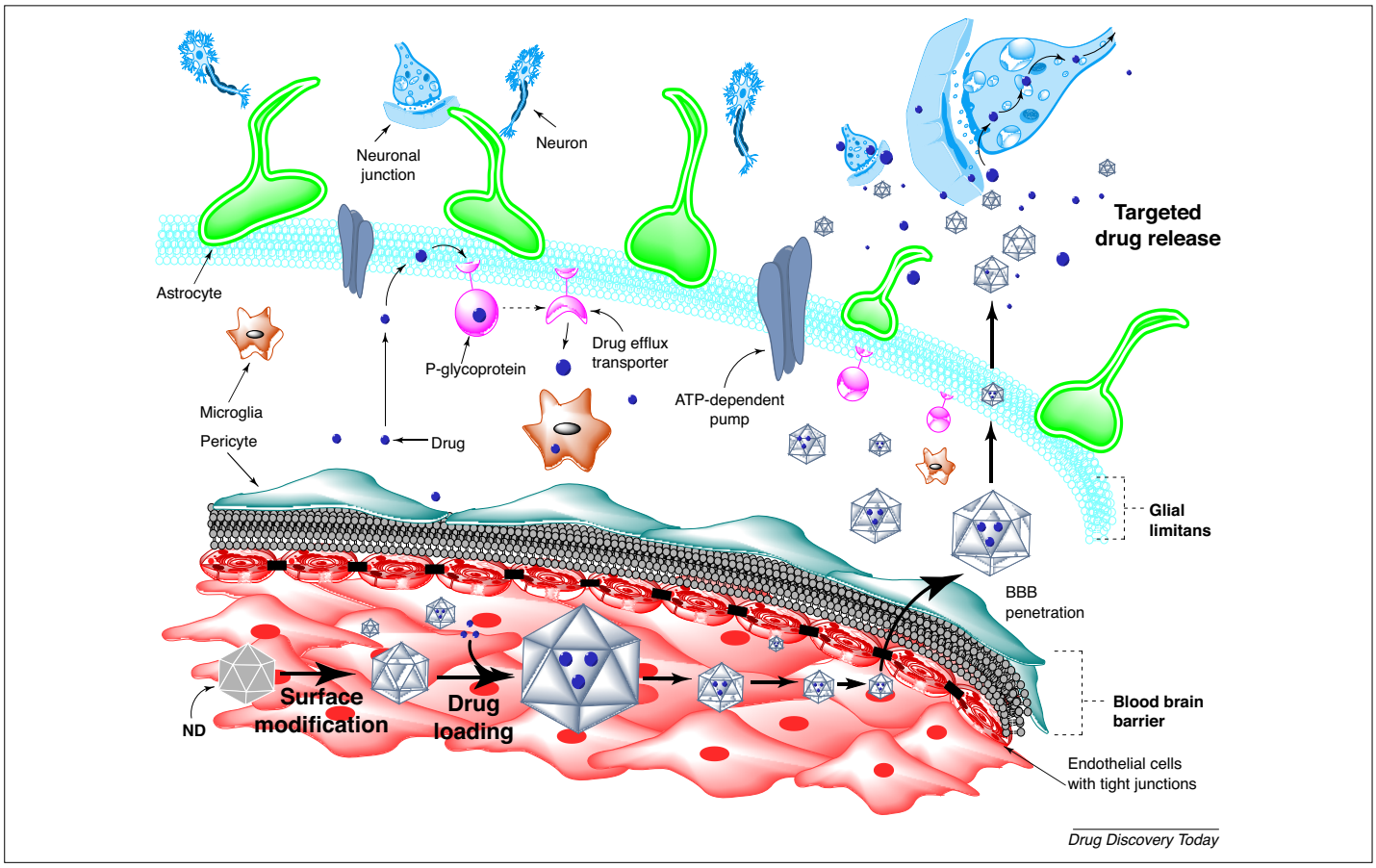

FIGURE 2

The use of nanodiamonds (NDs) as drug delivery systems (DDS) across the blood–brain barrier (BBB). Normally, delivering drugs across the BBB is difficult because intervening microglia and efflux transporters remove and prevent most drugs from entering the membrane, thereby reducing their efficacy. However, modification allows the conjugation of drugs to the ND surface, which then acts as a DDS. Its nanomolecular size allows it to evade microglia and efflux transporters and cross the BBB to reach its target site, where it can release the drug to show therapeutic effect.

with other molecules [62,63]. NDs are also 50 times as hard as titanium, which enables their use in medical devices including implants and surgical cutting tools [64]. ND coatings have been applied to temporomandibular joint prostheses, heart valves, and other biomedical microelectromechanical systems [65]. They have also been incorporated into bone tissue engineering, forming highly improved scaffolds facilitating bone mineralization and ossification as well as vascularization necessary for bone formation [66,67].

The inert nature of NDs also bestows on them a more significant safety profile. Chemically inert detonated NDs suitable for biomedical purposes are obtained by sequential purification processes, such as oxidation at high temperature in an air/ozone environment, to remove impurities adhered to the ND surface during synthesis [66,68]. The same purifying oxidation process also forms oxygen-containing functional groups (anhydrides and carboxylic acids) on the ND surface. Further air/ozone purification produces carboxylated NDs with highly reactive hydrophilic groups conducive for drug complexation [66]. This has driven *in vivo* research to establish their nontoxic profile [69]. With increasing knowledge of their biocompatibility, milled HPHT NDs gained considerable attention for their bioimaging applications [70]. Their intrinsic nitrogenous vacancies and sp^2 impurities bring about fluorescence with superior photostability voiding of the photobleaching effect that affects other fluorescent nanoprobes [71]. This optical property makes HPHT NDs excellent biomarking agents for diagnostic purposes.

Another exciting aspect of NDs is their surface tunability or the ability to modify the surfaces of these NPs as for maximum drug loading and site-specific delivery. By altering the surface microenvironment, NDs have great potential as a DDS [72] and sets them apart from other NPs because their carbon bonds and the truncated octahedral structure offer greater versatility. By modifying the surface, various functional groups can be created to which the drug can be complexed with and remain protected from the external environment during delivery. Biomolecules, such as DNA, RNA, and nucleic acids, have also been conjugated to ND surfaces, and their survival and functions were retained. Akiel *et al.* utilized a novel method of attaching single-stranded (ss)DNA to ND surfaces via site-directed spin labeling and paramagnetic resonance spectroscopy, retaining repetitive DNA hybridization and a high degree of mobility in conjunction with the ND [73]. Edgington *et al.* combined bead-assisted sonic deaggregation (BASD) with mixed silane (one negatively charged, the other functionally contributing amino groups) for *in situ* silanization and further covalent bonding with thiolated, ssDNA

oligomers [74]. The bonding between the ND and the molecule of interest forms a reliable DDS with higher specificity for the target and minimal drug leakage during delivery, thereby reducing the incidence of adverse effects [75].

The structure of NDs also provides a massive surface area to maximize adsorption capability. This allows higher drug loading and greater therapeutic efficacy. The adsorption/desorption phenomenon (through interactions between the functional groups on the ND surface and those of drugs) is crucial for the binding of drugs to the ND surface, retention during systemic circulation, uptake by target cells, and intracellular release for maximizing drug efficiency and minimizing systemic adverse effects of many antibiotics (vancomycin and tetracycline) and anticancer drugs (paclitaxel, doxorubicin, epirubicin, cisplatin, and camptothecin) [76–79]. The ability to adsorb nearly four times their weight in water enhances the hydrophilicity of NDs, allowing for more movement through bodily fluids [80]. This ability also enables these nanomaterials to behave like biosensors or biochips [81].

Chemotherapeutic NDs

NDs are already used as bioimaging agents and as drug carriers. They have shown promise in cancer theranostics by acting not only as contrast agents for tumor detection, but also as drug carriers to increase the therapeutic efficacy of a drug compared with conventional chemotherapy [82,83]. For example, NDs have been used to overcome the resistance and efflux of multidrug-resistant transporters that has hindered the use of other NPs. For instance, epirubicin showed nearly tenfold more therapeutic efficacy when NDs acted as drug carriers compared with its delivery by liposomes, which suffered high resistance and rejection by efflux transporters within cancer cells [84]. This ability to overcome the resistance of efflux transporters appears to be an asset more particularly associated with NDs than with other NPs, and such studies have repeatedly laid the foundation for utilizing this ability to also address neurological disorders.

The BBB poses a significant hurdle for neurotherapies because of resistance and poor efficacy leading to adverse effects [85]. There is also greater overexpression of efflux transporters, such as P-glycoprotein (a prominent ATP-binding cassette efflux pump in the BBB) during brain dysfunction, which appears to compromise the BBB [86]. In such cases, NDs might be able to enter the brain and efficiently deliver neuromedicine, thereby reduce adverse effects associated with poor bioavailability [87]. Various surface modifications and strategies have been implemented to enhance the delivery of neuromedicine through the BBB, but further studies are required to quantify ND concentration in the brain [52,63,88–90].

Brain tumors, such as glioblastomas, have a poor prognosis because of the BBB, reducing the survival time of patients to <1.5 years. Thus, doxorubicin, a cornerstone therapy for treating systemic neoplasms, has not been extensively utilized to treat brain malignancies because of its poor penetration of the BBB [91]. However, with the assistance of NDs, doxorubicin can not only cross the BBB to access brain tumors, but also be more efficacious. One study showcased the transmigration of a ND-doxorubicin complex through the BBB via convection enhanced delivery for treating glioblastoma [89]. A recent study by Chan *et al*. reported the versatility and surface tunability of NDs [78]. The authors modified NDs with dual ligands (folic acid and mitochondrial-localizing sequence peptides) to become highly specific for cancer cell mitochondria (Figs. 3 and 4 show proposed mechanisms by which NDs traverse the BBB to show therapeutic action).

Neuroprotection by nanodiamonds in neurodegenerative disorders

Most CNS disorders are characterized by the alteration and imbalance of various neurotransmitters and proteases [92–94]. It was recently shown that NDs can alter the velocity of neurotransmitter uptake and storage. Pozdnyakova *et al*. reported that NDs have the ability to be taken up by nerve terminals and to reduce the initial uptake and accumulation of the neurotransmitters glutamate and GABA [95] (Fig. 4). The authors observed a depolarizing effect by NDs, which affected sodium-dependent transporters responsible for neurotransmitter uptake. The study revealed the neuroactive properties of NDs that could be exploited for bioimaging of nerve terminals and to obtain a clearer understanding of the neurotransmitter processes in various CNS disorders involving abnormal neurotransmissions, such as epilepsy, schizophrenia, AD, AML, and PD.

Research into the genetic machinery underlying neurodegenerative diseases calls for highly sensitive detection techniques capable of detecting the subtle changes in gene expression [96]. As neurodegeneration progresses, different pathological states can be followed by studying the linked gene expressions [97]. Studies have corroborated the association between changes at the genetic level to anomalies observed during neuropathology [98,99]. Given the lack of methods sensitive enough to detect such elusive abnormalities, Haziza *et al*. developed a tracking technique utilizing the unique bioimaging ability of NDs to track gene expression during neurodegeneration [100]. By manipulating the uptake of fluorescent NDs (FNDs) into neurons, the authors observed the transit of NDs to investigate intraneuronal axonal transport within degrading neurons to understand better the genetic factors involved. Further expansion of this work could magnify the subtle protein changes at the center of each neuropathology and could help identify the crucial stages at which neurons exhibit the initial signs of disease for better diagnosis and more efficient prognosis. Given that most neurodegenerative diseases become active many years before their clinical manifestation, using biomarkers for early detection could significantly intervene in disease progression and preserve neuronal function [101].

Previous research pointed towards a link between aluminum exposure and AD [102]. Alawdi *et al*. investigated the neuroprotective role of NDs in rats with aluminum-induced AD-like cognitive deficits [103]. Aluminum upregulates nuclear factor kappa B (NF-κB), which translocates to the nucleus to trigger the transcription of proapoptotic and proinflammatory factors, such as interleukin (IL)-6 and IL-8. To exacerbate the pathology underlying AD, NF-κB also activates beta-amyloid cleavage enzyme 1 (BACE1), which hinders the clearance of β-amyloid 42 ($A\beta_{42}$) peptide monomers responsible for aggregating and forming β-amyloid (Aβ) plaques [104]. NF-κB also initiates the transcription of suppressor of cytokine signaling 3 (SOCS3), which inhibits Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) phosphorylation, which signals for apoptosis [105]. Thus, NF-κB appears to be at the center of many apoptotic signaling pathways responsible for neuronal cell death. It wa also reported

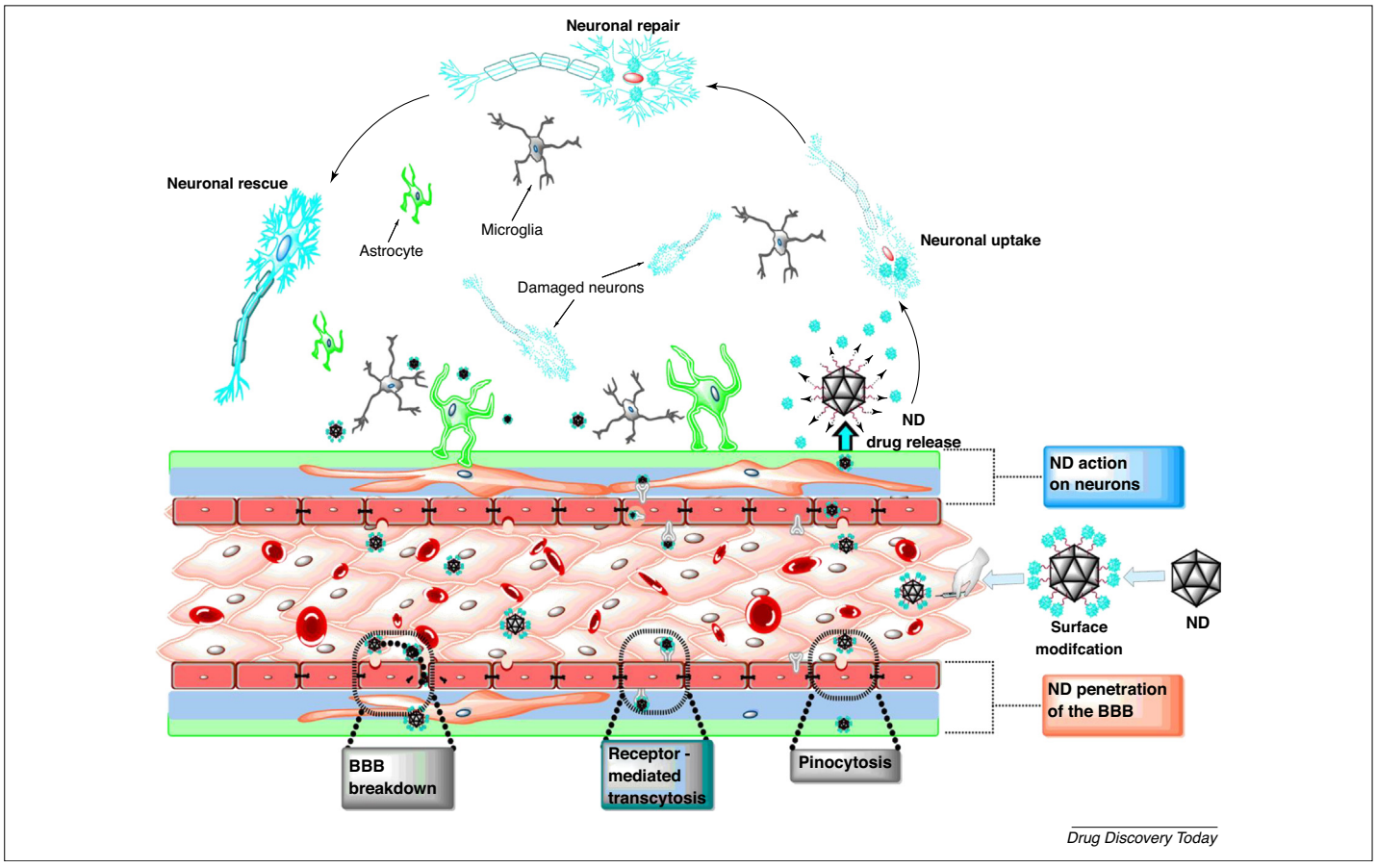

FIGURE 3

A probable mechanism of drug uptake by neurons and neuron salvaging. Nanodiamonds (NDs) can be surface modified and then injected into the systemic circulation to reach and traverse the blood–brain barrier (BBB) by methods such as BBB breakdown, receptor-mediated endocytosis, and pinocytosis. Once they have reached the target site, NDs can release the conjugated drug near the neuronal junctions, where it can show therapeutic action and salvage the remaining neuronal population.

that phosphorylated STAT3 induces anti-apoptotic factors, such Bcl-2 and Bcl-xL [106]. Alawdi *et al*. showed that NDs induced the upregulation of phosphorylated STAT3, which further downregulated NF-κB to enhance neuronal survival when exposed to aluminum [103]. NDs also stimulate brain-derived neurotrophic factor (BDNF), which is an essential neurotrophin that regulates neuronal growth, development, and maintenance [107]. Thus, this study showcases the intrinsic ability of NDs to interact and promote neuroprotection.

Interaction of NDs with neurons

The BBB protects neurons of the CNS. Therefore, if drug delivery systems are to be utilized to increase therapeutic efficacy, it is imperative that the drug carriers themselves are biocompatible with the neural machinery and do not contradict the therapeutic goal.

The effect of NDs on neurons was assessed by Huang *et al*. [108] by investigating the neuronal uptake of FNDs. The authors found no signs of toxicity in peripheral and CNS neurons *in vitro*. Although *in vivo* studies showed a dose-dependent reductive effect on neurite length, by comparing these results to those of a previous study in which neurite length increased [109], the authors saw the difference in particle size as being a factor and concluded that particle range of the NDs had a effect on neurite lengths: after a certain size threshold (possibly because of agglutination of NDs), there an inhibitory effect might result that causes a reduction in neurite growth. Thus, the smaller the ND, the more neuron outgrowth is observed. However, obtaining a small ND size distribution is often hindered by particle aggregation. Fortunately, methods such as salt-assisted ultrasonic deaggregation, zirconium-BASD coupled with carboxylation, and subjecting detonated ND hydrosols to hydrosol/glycerol centrifugation have been developed to obtain sharp distribution sizes of NDs in the 1–4-nm range [110–112].

Several NPs used as drug carriers cause neurotoxicity. In addition, the interaction with, and biocompatibility of, NDs in the CNS might also be cause for concern. Recent studies reported that NDs are biocompatible with neurons. NDs produced by the chemical vapor deposition method show the least amount of cytotoxicity and are excellent scaffolds for the differentiation and proliferation of neural stem cells (NSCs), although this method is expensive and not feasible for large-scale production, unlike HPHT [113]. The nanomaterials were so conducive to NSC survival that they resulted in greater populations of NSCs compared with those grown on polystyrene, which is a universal scaffold for culturing animal cells [114] (Fig. 5). Other studies reported the ordered growth and functionality of neurons after exposure to ND surfaces [115,116].

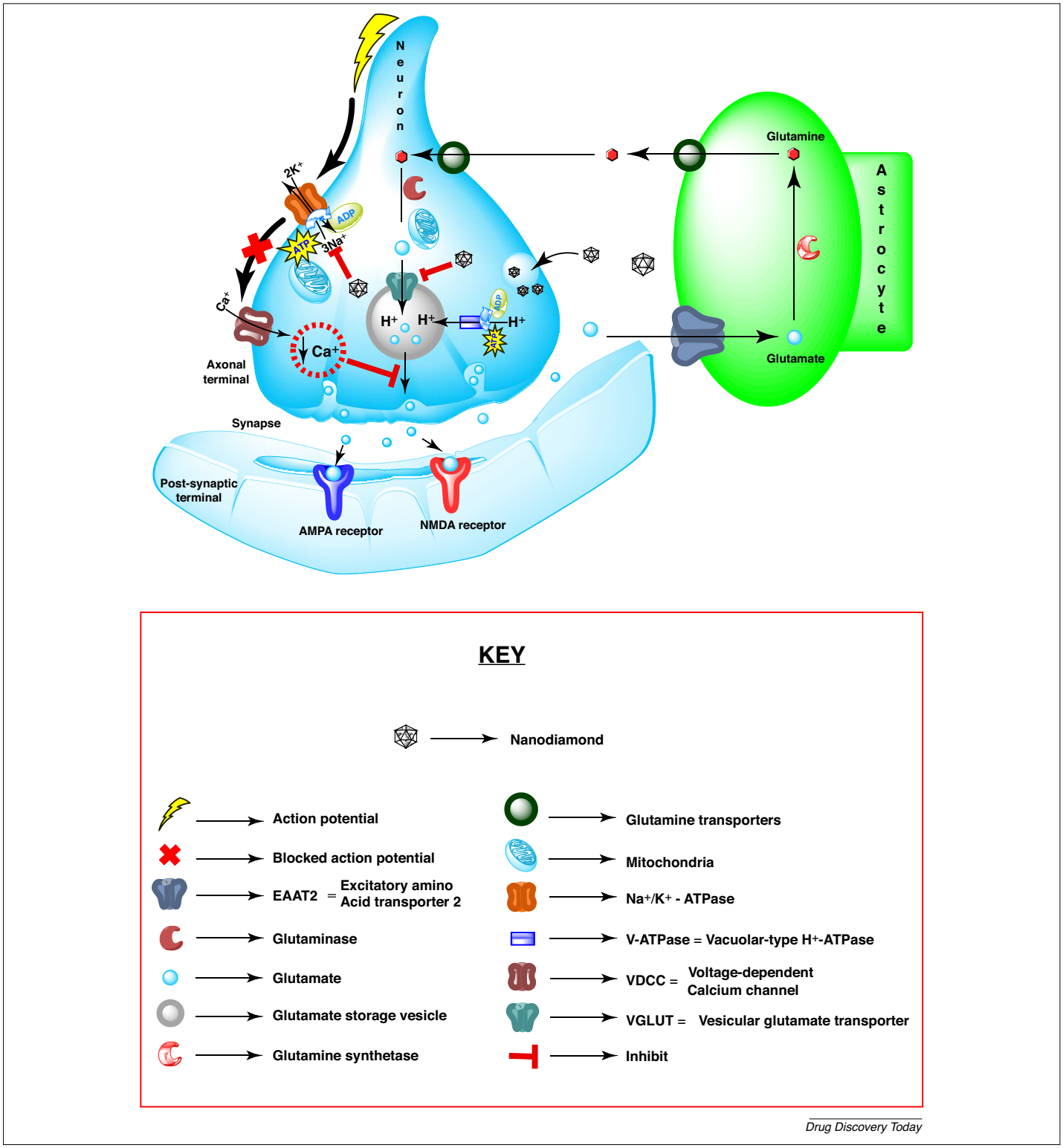

FIGURE 4

The ability of nanodiamonds (NDs) to alter and/or modulate neurotransmission signaling in neurons. Once taken up by neurons, NDs are proposed to influence and hinder the vesicular glutamate transporter (VGLUT) located within the axonal terminal and the sodium-potassium (NA^{+}–K^{+})-ATPase located on the membrane. This interference appears to reduce the amount of glutamate stored in vesicles as well as resulting in the decreased release of glutamate at the synapse. Therefore, NDs could curb the glutamate excitotoxicity often observed in many neurological disorders.

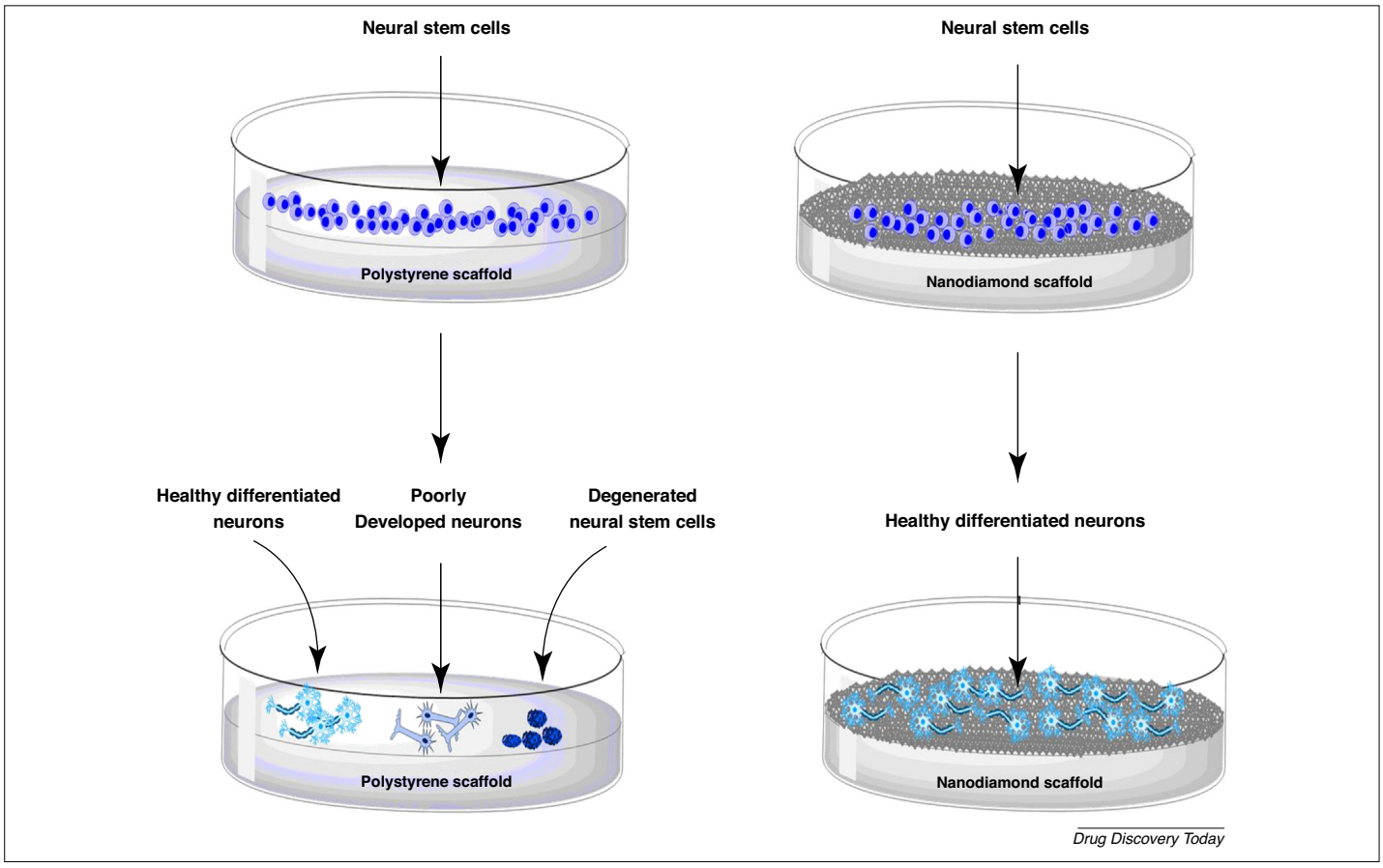

FIGURE 5

Greater survival, differentiation and proliferation of neural stem cells (NSCs) in response to nanodiamond (ND) culture compared with those grown on standard polystyrene scaffolds commonly used in cell cultures.

NDs for bioimaging and diagnosis

FNDs continue to flourish as biosensors. They are devoid of photobleaching, in which there is an alteration in the fluorophore resulting in the loss of fluorescent ability. FNDs contain negatively charged nitrogen vacancies (NV^-) formed as a result of high-bombardment radiation of HPHT NDs by neutrons, electrons, or ions. Other methods of producing FNDs (e.g., by modifying the surface chemistry of detonated NDs) have also emerged, although their photophysics are less understood and they are not feasible for large-scale production [117]. FNDs have an absorption maxima at ~550 nm (green–orange visible light) and an emission of ~700 nm lasting >10 ns [70]. They can be subjected to biological microscopy and can be detected by using fluorescence microscopy, optically detected magnetic resonance, and cathodoluminescence. FNDs serve as unique fluorophores capable of *in vivo* observation, and sustained and stable fluorescence during electron microscopy (EM) sample preparation, and also remain localized to provide excellent resolution [118]. Thus, FNDs have been used for exploring neural circuitry. Studies have documented the excellent biocompatibility between FNDs and neurons when labeling assessed their differentiation patterns with a monolayer of FNDs [119,120]. Morales-Zavala *et al.* showcased the superior fluorescence of FNDs compared with the commonly used markers thioflavin-T and fluorescein isothiocyanate (FITC) to detect biomarkers in AD [121]. FNDs were tagged to a bifunctional peptide (R7-CLPFFD) that is able to penetrate the endothelial lining (R7 is a hepta-arginine cell-penetrating peptide) and recognize $A\beta_{42}$ aggregates, a biomarker of AD. The study was performed on fibroblast cells and the bend.3 cell line (a brain vascular endothelial cell line). This photostability, along with their noncytotoxic nature, provides NDs with an excellent fluorescent property that enables them to act as ideal biosensors for use in tracking single particles within cells with high resolution and, thus, NDs hold great potential in the field of neural theranostics.

Concluding remarks

Here, we discussed the growing concern around neurodegenerative diseases and the challenges they present in the clinical setting. We have expounded on some of the current nanotechnologies available, their applications, and some of their shortcomings that paved the way for the development of NDs. NDs have features that are desirable for ideal NPs, including a nanoscale size range, inertness, biocompatibility, low to negligible toxicity, fluorescent ability devoid of photobleaching, and a massive surface area with high tunability to modify as needed. NDs are now in use as anticancer therapies and are slowly being used to address other pathologies. One particular area is neurodegeneration involved in many prominent neurological disorders, including AD, PD, HD, schizophrenia, ALS, and stroke. Given that current neurotherapies for these diseases only treat the symptoms but fail to be disease-

TABLE 1

Summary of the use of NDs in neurodegenerative disorders

Study	Study observations	Refs
Modified NDs as DDS for doxorubicin in treating glioblastomas	NDs penetrated BBB and enhanced efficacy of doxorubicin to treat glioblastomas	[78]
Effect of NDs on neurotransmitter uptake and storage	NDs reduced initial uptake and accumulation of glutamate and GABA through a depolarizing effect on sodium-dependent transporters involved in neurotransmitter uptake. This ability could be useful in studying bioimaging nerve terminals in different neurological disorders	[95]
Use of fluorescent NDs to study intraneuronal abnormalities during genetic changes in neurodegeneration	Transit of fluorescent NDs served as a marker and as a new technique to correlate genetic changes with intraneuronal axonal transport abnormalities	[100]
Effect of NDs on neurons in aluminum-induced AD-like neurodeficits	NDs upregulated phosphorylated STAT3, which in turn inhibited NF-κB, predominantly responsible for most of the apoptotic signaling during neurodegeneration caused by aluminum. NDs also influenced elevated BDNF expression. NDs showed an intrinsic ability to promote neuronal survival	[103,106,107]
Direct interaction between NDs and neurons	*In vitro* studies revealed no toxic effects of NDs on neurons. *In vivo* studies showcased a particle size-dependent reduction in neurite length. Smaller NDs elicited better neuronal outgrowth	[108,115,119]
Method of ND production and compatibility with NSCs	NDs produced by chemical vapor deposition method showed least amount of cytotoxicity and promoted NSC survival, differentiation, and proliferation compared with those grown on polystyrene	[114,116]
Sustained function of nucleic acids after conjugation to ND surface	Conjugation of DNA to ND surface maintained their functional integrity; thus, NDs could act as carriers of nucleic acids	[73]
NDs as bioimaging agents	Intrinsic fluorescent nature of NDs was used to track cellular components and processes	[100,122,123]

modifying, there is a need to investigate these diseases at the genetic level, which requires nanotechnologies capable of efficiently delivering therapeutic effects with minimum drug loss. NDs can successfully penetrate the BBB for efficient drug delivery with reduced drug loss, leading to fewer adverse effects. The concern of the biocompatibility between NDs and neurological tissue was assuaged after studies reported no cytotoxicity in neurons grown on ND-containing scaffolds. This has encouraged the use of NDs as bioimaging agents for neurological tumors and for studying neurotransmission processes via the endocytic uptake of NDs by neurons. More recent studies elicited the positive therapeutic effect of NDs themselves because they showed antiapoptotic effects and neuroprotection against aluminum-induced neurodegeneration (Table 1).

Despite this progress, studies involving NDs for biomedical research face several hurdles, such as the difficulties in fabrication (high energy demand and operating temperatures, low yields, and high costs), purification, and obtaining sharp distribution sizes. Furthermore, a greater understanding of their aggregation behavior as well as their interaction with other molecules and interfaces (such as the BBB and efflux transporters) requires more research. These are complex conundrums plaguing diamond material research for biomedical applications because such research is still in its infancy. Yet, the versatility offered by NDs is promising and will only spark further research into NDs as the ideal DDS to the brain. With their good safety profile, efficient drug delivery, and an almost endless array of surface modifications, NDs certainly warrant further studies as treatments for neurodegenerative diseases and could offer an exciting option for neurotherapy in the future.

Acknowledgments

The authors thank the Department of Pharmaceuticals, Ministry of Chemicals and Fertilizers for financial support. R.K.T. thanks the Science and Engineering Research Board (Statutory Body Established Through an Act of Parliament: SERB Act 2008), Department of Science and Technology, Government of India for a grant (#ECR/2016/001964) and N-PDF funding (PDF/2016/003329). P.B. thanks SERB, Department of Science and Technology (DST), Government of India for financial support (Grant SB/YS/LS-196/2014) and an International Society for Neurochemistry (ISN) Return Home grant.

References

1 United Nations Department of Economicand Social Affairs (2015) *World Population Prospects: The 2015 Revision, Key Findings and Advance Tables*. United Nations Department of Economic and Social Affairs
2 Chin, J.H. and Vora, N. (2014) The global burden of neurologic diseases. *Neurology* 83, 349–351
3 Murray, C.J. *et al.* (2012) Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions (1990–2010: a systematic analysis for the Global Burden of Disease Study 2010). *Lancet* 380, 2197–2223
4 Przedborski, S. *et al.* (2003) Series Introduction: Neurodegeneration: what is it and where are we? *J. Clin. Invest.* 11, 3–10
5 Wyss-Coray, T. (2016) Ageing, neurodegeneration and brain rejuvenation. *Nature* 539, 180
6 Brown, R.C. *et al.* (2005) Neurodegenerative diseases: an overview of environmental risk factors. *Environ. Health Perspect.* 113, 1250
7 Williams, A. (2002) Defining neurodegenerative diseases: disorders will be named after responsible rogue proteins and their solutions. *BMJ* 324, 1465
8 Blennow, K. *et al.* (2010) Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat. Rev. Neurol.* 6, 131
9 Shaw, L.M. *et al.* (2007) Biomarkers of neurodegeneration for diagnosis and monitoring therapeutics. *Nat. Rev. Drug Discov.* 6 nrd2176
10 Page, T.E. *et al.* (2017) Instruments measuring the disease-specific quality of life of family carers of people with neurodegenerative diseases: a systematic review. *BMJ Open* 7, e013611
11 Schrag, A. *et al.* (2000) What contributes to quality of life in patients with Parkinson's disease? *J. Neurol. Neurosurg. Psychiatry* 69, 308–312
12 do Carmo Costa, M. and Paulson, H.L. (2013) New hope for therapy in neurodegenerative diseases. *Cell Res.* 23, 1159
13 Ali, I.U. and Chen, X. (2015) Penetrating the blood–brain barrier: promise of novel nanoplatforms and delivery vehicles. *ACS Nano* 9, 9470–9474
14 Brightman, M. and Reese, T. (1969) Junctions between intimately apposed cell membranes in the vertebrate brain. *J. Cell Biol.* 40, 648–677
15 Kabanov, A. and Gendelman, H. (2007) Nanomedicine in the diagnosis and therapy of neurodegenerative disorders. *Prog. Polym. Sci.* 32, 1054–1082
16 Pardridge, W.M. (2009) Alzheimer's disease drug development and the problem of the blood–brain barrier. *Alzheimers Demen.* 5, 427–432
17 Lansbury, P.T., Jr (2004) Back to the future: the 'old-fashioned' way to new medications for neurodegeneration. *Nat. Med.* 10, S51
18 Lu, B. *et al.* (2013) BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. *Nat. Rev. Neurosci.* 14, 401
19 Wang, T. *et al.* (2012) Cellular uptake of nanoparticles by membrane penetration: a study combining confocal microscopy with FTIR spectroelectrochemistry. *ACS Nano* 6, 1251–1259
20 Bhaskar, S. *et al.* (2010) Multifunctional Nanocarriers for diagnostics, drug delivery and targeted treatment across blood–brain barrier: perspectives on tracking and neuroimaging. *Particle Fibre Toxicol.* 7, 3
21 Bruchez, M. *et al.* (1998) Semiconductor nanocrystals as fluorescent biological labels. *Science* 281, 2013–2016
22 Chan, W.C. and Nie, S. (1998) Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science* 281, 2016–2018
23 Kato, D. and Oishi, M. (2014) Ultrasensitive detection of DNA and RNA based on enzyme-free click chemical ligation chain reaction on dispersed gold nanoparticles. *ACS Nano* 8, 9988–9997
24 Chinen, A.B. *et al.* (2015) Nanoparticle probes for the detection of cancer biomarkers, cells, and tissues by fluorescence. *Chem. Rev.* 15, 10530–10574
25 Espinosa, A. *et al.* (2016) Duality of iron oxide nanoparticles in cancer therapy: amplification of heating efficiency by magnetic hyperthermia and photothermal bimodal treatment. *ACS Nano* 10, 2436–2446
26 Parak, W.J. *et al.* (2002) Cell motility and metastatic potential studies based on quantum dot imaging of phagokinetic tracks. *Adv. Mater.* 14, 882–885
27 Chen, G. *et al.* (2016) Nanochemistry and nanomedicine for nanoparticle-based diagnostics and therapy. *Chem. Rev.* 116, 2826–2885
28 Decuzzi, P. *et al.* (2010) Size and shape effects in the biodistribution of intravascularly injected particles. *J. Control. Release* 141, 320–327
29 Bramini, M. *et al.* (2014) Imaging approach to mechanistic study of nanoparticle interactions with the blood-brain barrier. *ACS Nano* 8, 4304–4312
30 Smith, N.M. *et al.* (2016) An unexpected transient breakdown of the blood brain barrier triggers passage of large intravenously administered nanoparticles. *Sci. Rep.* 6, 22595
31 Blanco, E. *et al.* (2015) Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat. Biotechnol.* 33, 941
32 Guerrero, S. *et al.* (2010) Improving the brain delivery of gold nanoparticles by conjugation with an amphipathic peptide. *Nanomedicine* 5, 897–913
33 Choi, C.H.J. *et al.* (2010) Mechanism of active targeting in solid tumors with transferrin-containing gold nanoparticles. *Proc. Natl. Acad. Sci. U. S. A.* 107, 1235–1240
34 Zhang, L. *et al.* (2011) Rutile TiO_2 particles exert size and surface coating dependent retention and lesions on the murine brain. *Toxicol. Lett.* 207, 73–81
35 Chen, J.-K. *et al.* (2015) Particulate nature of inhaled zinc oxide nanoparticles determines systemic effects and mechanisms of pulmonary inflammation in mice. *Nanotoxicology* 9, 43–53
36 Tekade, R.K. *et al.* (2008) Dendrimers in oncology: an expanding horizon. *Chem. Rev.* 109, 49–87
37 Stasko, N.A. *et al.* (2007) Cytotoxicity of polypropylenimine dendrimer conjugates on cultured endothelial cells. *Biomacromolecules* 8, 3853–3859
38 Chang, H. *et al.* (2014) Surface-engineered dendrimers with a diaminododecane core achieve efficient gene transfection and low cytotoxicity. *Bioconjug. Chem.* 25, 342–350
39 Dwivedi, R. *et al.* (2013) Nanoparticulate carrier mediated intranasal delivery of insulin for the restoration of memory signaling in Alzheimer's disease. *Curr. Nanosci.* 9, 46–55
40 Gandhi, N.S. *et al.* (2014) Nanocarrier mediated delivery of siRNA/miRNA in combination with chemotherapeutic agents for cancer therapy: current progress and advances. *J. Control. Release* 194, 238–256
41 Liu, Y. *et al.* (2012) Understanding the toxicity of carbon nanotubes. *Acc. Chem. Res.* 46, 702–713
42 Shin, S.W. *et al.* (2015) Role of physicochemical properties in nanoparticle toxicity. *Nanomaterials* 5, 1351–1365
43 Yu, S.-J. *et al.* (2005) Bright fluorescent nanodiamonds: no photobleaching and low cytotoxicity. *J. Am. Chem. Soc.* 127, 17604–17605
44 Paget, V. *et al.* (2014) Carboxylated nanodiamonds are neither cytotoxic nor genotoxic on liver, kidney, intestine and lung human cell lines. *Nanotoxicology* 8, 46–56
45 Zupančič, D. *et al.* (2018) Detonation nanodiamonds are promising nontoxic delivery system for urothelial cells. *Protoplasma* 255, 419–423
46 Wa?sowicz, M. *et al.* (2017) Haemocompatibility of modified nanodiamonds. *Materials* 10, 352
47 Chow, E.K. *et al.* (2011) Nanodiamond therapeutic delivery agents mediate enhanced chemoresistant tumor treatment. *Sci. Transl. Med.* 3, 73ra21
48 Pichot, V. *et al.* (2013) Understanding ultrafine nanodiamond formation using nanostructured explosives. *Sci. Rep.* 3, 2159
49 Tetienne, J.-P. *et al.* (2013) Spin relaxometry of single nitrogen-vacancy defects in diamond nanocrystals for magnetic noise sensing. *Phys. Rev. B* 87, 235436
50 Ong, S. *et al.* (2017) Shape and crystallographic orientation of nanodiamonds for quantum sensing. *Phys. Chem. Chem. Phys.* 19, 10748–10752
51 Ansari, S.A. *et al.* (2016) Role of nanodiamonds in drug delivery and stem cell therapy. *Iran. J. Biotechnol.* 14, 130
52 Zhang, B. *et al.* (2017) Anchored but not internalized: shape dependent endocytosis of nanodiamond. *Sci. Rep.* 7, 46462
53 Vácha, R. *et al.* (2011) Receptor-mediated endocytosis of nanoparticles of various shapes. *Nano Lett.* 11, 5391–5395
54 Canton, I. and Battaglia, G. (2012) Endocytosis at the nanoscale. *Chem. Soc. Rev.* 41, 2718–2739
55 Albanese, A. *et al.* (2012) The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annu. Rev. Biomed. Eng.* 14, 1–16
56 Mochalin, V.N. *et al.* (2012) The properties and applications of nanodiamonds. *Nat. Nanotechnol.* 7, 11
57 Danilenko, V. (2004) *On the History of the Discovery of Nanodiamond Synthesis*. Springer
58 Nee, C.-H. *et al.* (2016) Direct synthesis of nanodiamonds by femtosecond laser irradiation of ethanol. *Sci. Rep.* 6, 33966
59 Boudou, J.-P. *et al.* (2009) High yield fabrication of fluorescent nanodiamonds. *Nanotechnology* 20, 235602
60 Hoffman, E.N. *et al.* (2005) Synthesis of carbide-derived carbon by chlorination of Ti2AlC. *Chem. Mater.* 17, 2317–2322
61 Gorrini, F. *et al.* (2016) On the thermodynamic path enabling a room-temperature, laser-assisted graphite to nanodiamond transformation. *Sci. Rep.* 6, 35244
62 Barnard, A.S. (2018) Predicting the impact of structural diversity on the performance of nanodiamond drug carriers. *Nanoscale* 10, 8893–8910
63 van der Laan, K. *et al.* (2018) Nanodiamonds for in vivo applications. *Small* 14, 1703838

64 Huang, Q. *et al.* (2014) Nanotwinned diamond with unprecedented hardness and stability. *Nature* 510, 250
65 Narayan, R.J. *et al.* (2011) Medical applications of diamond particles & surfaces. *Mater. Today* 14, 154–163
66 Aversa, R. *et al.* (2017) Nano-diamond hybrid materials for structural biomedical application. *Am. J. Biochem. Biotechnol.* 13, 34–41
67 Wu, X. *et al.* (2017) Functionalization of bone implants with nanodiamond particles and angiopoietin-1 to improve vascularization and bone regeneration. *J. Mater. Chem. B* 5, 6629–6636
68 Tzeng, Y. *et al.* (2014) Nitrogen-incorporated ultrananocrystalline diamond and multi-layer-graphene-like hybrid carbon films. *Sci. Rep.* 4, 4531
69 Mohan, N. *et al.* (2010) In vivo imaging and toxicity assessments of fluorescent nanodiamonds in Caenorhabditis elegans. *Nano Lett.* 10, 3692–3699
70 Hsiao, W.W.-W. *et al.* (2016) Fluorescent nanodiamond: a versatile tool for long-term cell tracking, super-resolution imaging, and nanoscale temperature sensing. *Acc. Chem. Res.* 49, 400–407
71 Mohan, N. *et al.* (2010) Sub-20-nm fluorescent nanodiamonds as photostable biolabels and fluorescence resonance energy transfer donors. *Adv. Mater.* 22, 843–847
72 Zhang, X.Q. *et al.* (2011) Multimodal nanodiamond drug delivery carriers for selective targeting, imaging, and enhanced chemotherapeutic efficacy. *Adv. Mater.* 23, 4770–4775
73 Akiel, R.D. *et al.* (2016) Investigating functional DNA grafted on nanodiamond surface using site-directed spin labeling and electron paramagnetic resonance spectroscopy. *J. Phys. Chem. B* 120, 4003–4008
74 Edgington, R. *et al.* (2018) Functionalisation of detonation nanodiamond for monodispersed, soluble DNA-nanodiamond conjugates using mixed silane bead-assisted sonication disintegration. *Sci. Rep.* 8, 728
75 Ho, D. *et al.* (2015) Nanodiamonds: the intersection of nanotechnology, drug development, and personalized medicine. *Sci. Adv.* 1, e1500439
76 Giammarco, J. *et al.* (2016) The adsorption of tetracycline and vancomycin onto nanodiamond with controlled release. *J. Colloid Interface Sci.* 468, 253–261
77 Lim, D.G. *et al.* (2016) Paclitaxel-nanodiamond nanocomplexes enhance aqueous dispersibility and drug retention in cells. *ACS Appl. Mater. Interfaces* 8, 23558–23567
78 Chan, M.S. *et al.* (2017) Cancer-cell-specific mitochondria-targeted drug delivery by dual-ligand-functionalized nanodiamonds circumvent drug resistance. *ACS Appl. Mater. Interfaces* 9, 11780–11789
79 Yu, Y. *et al.* (2018) Self-assembled nanodiamond supraparticles for anticancer chemotherapy. *Nanoscale* 10, 8969–8978
80 Petit, T. *et al.* (2015) Probing interfacial water on nanodiamonds in colloidal dispersion. *J. Phys. Chem. Lett.* 6, 2909–2912
81 Zhang, W. *et al.* (2014) Nanostructuring of biosensing electrodes with nanodiamonds for antibody immobilization. *ACS Nano* 8, 1419–1428
82 Hou, W. *et al.* (2017) Nanodiamond-manganese dual mode MRI contrast agents for enhanced liver tumor detection. *Nanomedicine* 13, 783–793
83 Rammohan, N. *et al.* (2016) Nanodiamond-gadolinium (iii) aggregates for tracking cancer growth in vivo at high field. *Nano Lett.* 16, 7551–7564
84 Wang, X. *et al.* (2014) Epirubicin-adsorbed nanodiamonds kill chemoresistant hepatic cancer stem cells. *ACS Nano* 8, 12151–12166
85 Sisodiya, S. *et al.* (2002) Drug resistance in epilepsy: expression of drug resistance proteins in common causes of refractory epilepsy. *Brain* 125, 22–31
86 Abbott, N.J. *et al.* (2006) Astrocyte–endothelial interactions at the blood–brain barrier. *Nat. Rev. Neurosci.* 7, 41
87 Liu, J.Y. *et al.* (2012) Neuropathology of the blood-brain barrier and pharmaco-resistance in human epilepsy. *Brain* 135, 3115–3133
88 Takayama, K. *et al.* (2009) Novel system to achieve one-pot modification of cargo molecules with oligoarginine vectors for intracellular delivery. *Bioconjug. Chem.* 20, 249–257
89 Xi, G. *et al.* (2014) Convection-enhanced delivery of nanodiamond drug delivery platforms for intracranial tumor treatment. *Nanomedicine* 10, 381–391
90 Joliot, A. and Prochiantz, A. (2004) Transduction peptides: from technology to physiology. *Nat. Cell Biol.* 6, 189
91 Stupp, R. *et al.* (2009) Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol.* 10, 459–466
92 Palmer, A.M. *et al.* (1988) Possible neurotransmitter basis of behavioral changes in Alzheimer's disease. *Ann. Neurol.* 23, 616–620
93 Spehlmann, R. and Stahl, S. (1976) Dopamine acetylcholine imbalance in Parkinson's disease: possible regenerative overgrowth of cholinergic axon terminals. *Lancet* 307, 724–726
94 Bhattacharya, P. *et al.* (2014) Alleviation of glutamate mediated neuronal insult by piroxicam in rodent model of focal cerebral ischemia: a possible mechanism of GABA agonism. *J. Physiol. Biochem.* 70, 901–913
95 Pozdnyakova, N. *et al.* (2016) Neuroactivity of detonation nanodiamonds: dose-dependent changes in transporter-mediated uptake and ambient level of excitatory/inhibitory neurotransmitters in brain nerve terminals. *J. Nanobiotechnol.* 14, 25
96 McClellan, J. and King, M.-C. (2010) Genetic heterogeneity in human disease. *Cell* 141, 210–217
97 Cooper-Knock, J. *et al.* (2012) Gene expression profiling in human neurodegenerative disease. *Nat. Rev. Neurol.* 8, 518
98 Hirokawa, N. *et al.* (2010) Molecular motors in neurons: transport mechanisms and roles in brain function, development, and disease. *Neuron* 68, 610–638
99 Millecamps, S. and Julien, J.-P. (2013) Axonal transport deficits and neurodegenerative diseases. *Nat. Rev. Neurosci.* 14, 161
100 Haziza, S. *et al.* (2017) Fluorescent nanodiamond tracking reveals intraneuronal transport abnormalities induced by brain-disease-related genetic risk factors. *Nat. Nanotechnol.* 12, 322
101 DeKosky, S.T. and Marek, K. (2003) Looking backward to move forward: early detection of neurodegenerative disorders. *Science* 302, 830–834
102 Altmann, P. *et al.* (1999) Disturbance of cerebral function in people exposed to drinking water contaminated with aluminium sulphate: retrospective study of the Camelford water incident. *BMJ* 319, 807–811
103 Alawdi, S.H. *et al.* (2017) Neuroprotective effect of nanodiamond in Alzheimer's disease rat model: a pivotal role for modulating NF-κB and STAT3 signaling. *Mol. Neurobiol.* 54, 1906–1918
104 Zhao, Y. *et al.* (2014) Aluminum-induced amyloidogenesis and impairment in the clearance of amyloid peptides from the central nervous system in Alzheimer's disease. *Front. Neurol.* 5, 167
105 Yoshimura, A. *et al.* (2007) SOCS proteins, cytokine signalling and immune regulation. *Nat. Rev. Immunol.* 7, 454
106 Siddiquee, K.A.Z. and Turkson, J. (2008) STAT3 as a target for inducing apoptosis in solid and hematological tumors. *Cell Res.* 18, 254
107 Nagahara, A.H. *et al.* (2009) Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. *Nat. Med.* 15, 331
108 Huang, Y.-A. *et al.* (2014) The effect of fluorescent nanodiamonds on neuronal survival and morphogenesis. *Sci. Rep* 4, 6919
109 Edgington, R. *et al.* (2013) Patterned neuronal networks using nanodiamonds and the effect of varying nanodiamond properties on neuronal adhesion and outgrowth. *J. Neural Eng.* 10, 056022
110 Turcheniuk, K. *et al.* (2016) Salt-assisted ultrasonic deaggregation of nanodiamond. *ACS Appl. Mater. Interfaces* 8, 25461–25468
111 Pedroso-Santana, S. *et al.* (2017) Deagglomeration and characterization of detonation nanodiamonds for biomedical applications. *J. Appl. Biomed.* 15, 15–21
112 Trofimuk, A. *et al.* (2018) Effective method for obtaining the hydrosols of detonation nanodiamond with particle size <4 nm. *Materials* 11, 1285
113 Nian, Q. *et al.* (2014) Direct laser writing of nanodiamond films from graphite under ambient conditions. *Sci. Rep.* 4, 6612
114 Chen, Y.-C. *et al.* (2009) The effect of ultra-nanocrystalline diamond films on the proliferation and differentiation of neural stem cells. *Biomaterials* 30, 3428–3435
115 Specht, C.G. *et al.* (2004) Ordered growth of neurons on diamond. *Biomaterials* 25, 4073–4078
116 Ruiz, A. *et al.* (2008) Micro-stamped surfaces for the patterned growth of neural stem cells. *Biomaterials* 29, 4766–4774
117 Reineck, P. *et al.* (2017) Effect of surface chemistry on the fluorescence of detonation nanodiamonds. *ACS Nano* 11, 10924–10934
118 Hemelaar, S. *et al.* (2017) Nanodiamonds as multi-purpose labels for microscopy. *Sci. Rep.* 7, 720
119 Thalhammer, A. *et al.* (2010) The use of nanodiamond monolayer coatings to promote the formation of functional neuronal networks. *Biomaterials* 31, 2097–2104
120 Hsu, T.-C. *et al.* (2014) Labeling of neuronal differentiation and neuron cells with biocompatible fluorescent nanodiamonds. *Sci. Rep.* 4, 5004
121 Morales-Zavala, F. *et al.* (2018) Functionalization of stable fluorescent nanodiamonds towards reliable detection of biomarkers for Alzheimer's disease. *J. Nanobiotechnol.* 16, 60
122 Chang, Y.-R. *et al.* (2008) Mass production and dynamic imaging of fluorescent nanodiamonds. *Nat. Nanotechnol.* 3, 284
123 Fu, C.-C. *et al.* (2007) Characterization and application of single fluorescent nanodiamonds as cellular biomarkers. *Proc. Natl. Acad. Sci. U. S. A.* 104, 727–732