



NeuroPharmacology

Review Article

Revisiting Neuroscience with Nanomedicine, a Renaissance in Remedy

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Abstract

Central nervous system disorders including epilepsy, spinal cord injury, schizophrenia, Parkinson's and Alzheimer's disease, and other more potent gliomas lead to severe and fatal difficulties in day today lives. The existing therapies aim to improve the functional capacity of the patient for as long as possible; however, they do not modify the progression of the underlying neuropathological process. The need for newer and more effective targeting is consequently receiving a great deal of attention in order to overcome poor efficacy and safety issues associated with investigational agents in clinical trials. Due to comprehensive conclusion of the disease etiologies and suitable modification of the pathological process by nanoplateforms, yesterday's incurable ailments are going to turn curable tomorrow. Recent advances in nanotechnology have improved the ability to specifically tailor the features and properties of nanoparticles and nanodevises for biomedical applications, especially in reaching those targets, remote to the existing remedial technologies. Nanomedicine is a budding area of research that includes the application of these nanotechnological aspects to medicine. This "little big" science usually encompass miniaturization of devices; imaging techniques, drug delivery systems, nano-sized materials, and other novel bio-analytical tools that could aid in better understanding of disease pathophysiology and mitigation neuronal abnormalities. This review provides an overview of the most recent research and applications of new nanotechniques and/or nanomaterials that are

envisaged to have a major impact on the diagnosis and therapy of various neuronal disorders. Moreover, it also addresses the future prospects of nanomedicine in neuropharmacology, and project possible challenges for the nano- and neuro-scientists for effective use of nanomaterials to culminate in their safe use.



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Introduction

“The problems of chemistry and biology can be greatly helped if our (physicists’) ability to see what we are doing, and to do things on an atomic level, is ultimately developed-- a development which I think cannot be avoided”

--- Richard P. Feynman, 29th December, 1959

It is over half a century ago when the concept of nanoscience was put forward and since then the research in this field is rapidly mounting in varied areas of science and technology. On the other hand, considerable number of review articles related to nanoscience appeared only after 1990, with particular focus on physical and surface chemistry, materials science and instrumentation, but only a fraction of the papers included studies of nanotechnology in relation to medicine in general, and to neuroscience in particular. If one does a bibliographic research using the key word ‘nanotechnology or nanoscience’ and ‘neuroscience or brain’, one detects around 100 review titles. Of this total, about two-third is related to the research in drug delivery systems to transit blood brain barrier. In this context, nanoscience and nanotechnology are pervading enormously into the arena of neuroscience and many projects are being taken up to harbor therapeutic outputs of nanoscience research to diverse neuropathological conditions, which warrant critical attention.

At this current scenario the nanotechnology needs to be extensively studied and maneuvered to accomplish maximum benefits in ameliorating neuronal disease states and in rectifying brain dysfunction. Nanomedicine regarded to be an outcome of extensive research in interdisciplinary areas including physical, chemical, systems biology and medicine percolated the advanced scientific benefits for the manipulation and mitigation of the neuronal ailments and associated abnormalities. Nanomedicine particularly includes imaging, diagnosis, drug delivery, tissue regeneration, and production of novel medical devises. The nanomaterials such as liposomes, dendrimers, nanotubes, nanofibers and quantum dots which fall in the locus of nanomedicine are capable of interacting with biological systems. These can be tailored so as to respond to specific cell environments and even to induce desired physiological responses, while minimizing untoward effects.

This is the first review of this type which has mended the recent advances in the nanotechnology field to most of the medical conditions especially related to brain. This review gives a detailed description of the application of various nano-processes in central nervous

system disorders. Moreover, this article presents an overview of the future prospects, and discusses the issues, approaches and challenges, with the aim of improving the concept of nanomedicine in the neuroscience research and development. A brief introduction to basic aspects of neuroscience and nanoscience in whole will be made at the beginning to facilitate understanding by readers new to neuro and/or nanoscience disciplines.

Neuroscience and its limitations

Neuroscience relates to the study of the nervous system, broadly classified as central nervous system (CNS) which includes brain and spinal cord and peripheral nervous system made up of the nerves that serve the neck and arms, trunk, legs, skeletal muscles and internal organs. Brain is the vital and foremost organ of the human body and is made up of billions of neurons, the functional units of the nervous system. Non-neuronal cells of the brain include glia which provides structural and metabolic support, insulation, and participate in signal transmission of neurons. Neurons communicate with each other by transmitting electrical signals for long distances and then releasing chemicals called neurotransmitters which cross complex knots known as neuronal junctions or synapses (Fig.1). In recent decades neuroscience became a daunting frontier which integrates biology, chemistry, and physics with studies of structure, physiology, and behavior, including human emotional and cognitive functions. The current neuroscience research holds great promise for understanding and treating several neuronal disorders including Parkinson's disease, Alzheimer's disease, stroke, epilepsy, schizophrenia, etc.

These neuronal disorders constitute the major mainstay of the ailments suffered by humans in par with the metabolic and cardiovascular anachronisms. As the available medication can manage only symptoms, not the disease process, the search for the effective means for diagnosis and therapy is still surging. On the other hand, the most challenging aspect in the therapy targeting CNS is the complex circuitry of the anatomical structures of the brain which provide limited accesses to the xenobiotics. Penetration of substances into the brain is limited by the blood brain barrier (BBB) that is formed by the endothelium of brain vessels, the basal membrane, and neuroglial cells (Fig.1). The border of the BBB has a characteristic, tight continuous junction between the endothelial cells that prevents transport between these cells and restricts the uptake of substances into the brain based on molecular size and lipophilicity. In fact, only lipophilic, low molecular weight and unionized molecules at physiological pH, can diffuse freely through the endothelial membrane and may passively cross the BBB. Large, water-soluble molecules are mainly prohibited from entering the brain whereas small, lipophilic particles can penetrate the BBB from the plasma more liberally. However, a large number of drugs that are favorably lipophilic and normally presumed to exhibit an easy transit across endothelial cells are

rapidly pumped back into the bloodstream by effective efflux pump systems [1]. These efflux pump systems include multiple organic anion transporters and P-glycoprotein, sometimes referred to as multidrug resistance protein. Other essential compounds such as amino acids, hexoses, neuropeptides, and proteins traverse the BBB through specific carriers or transporter systems. Consequently, this preferential barrier between the brain and its surroundings environment facilitates the maintenance of the internal milieu of the brain to ensure homeostasis, but at the same time, fosters risk for distribution of drugs and thus compromise the efficient treatment of neuronal and psychiatric disorders.

Molecular imaging is a powerful method for animal models of disease and is also being used extensively in clinical investigations as a non-invasive means for monitoring disease progress and response to therapeutics [2]. Despite yielding the highest resolution (down to ~0.2 nm) of all imaging techniques, electron microscopy has a major drawback that it remains only applicable to fixed/dehydrated samples, as proper function of an electron microscope requires the material of interest to be maintained in a vacuum. Moreover, labeling of cells and organelles, for electron microscopy requires staining with electron-dense metal ions such as uranyl acetate or lead citrate [3]. The existing limitations in the imaging techniques and other bio-analytical methods also lingers the generation of effective therapy for these CNS disorders. Combination of precise targeting using specific antibodies and imaging enhancement properties of nanoparticles are the key to greatly enhance the power of various imaging techniques.

Nanoscience, nanotechnology and nanomedicine as they unfold

Nanoscience is acknowledged to be a new frontier of research in science and technology of the 21st century. With the advent of the novel technologies, and rapid strides in the interdisciplinary research including basic (such as biology, chemistry, mathematics and physics) and applied fields (such as materials science and various areas of engineering) the novel concept of nanoscience was emerged with enormous potential in increasing the life expectancy of suffering humans. Starting from the fabrication of atomic force microscopy to the laser ablation coupled plasma mass spectrometry [4], the volume of the application of the nanotechnology have resulted in the nanometer-scale analysis of elements on sample surfaces and produced efficient and sensitive trace and surface analytical techniques for visualizing cells and tissues.

The conceptualization of research at the nanoscale is generally considered to be materialized ever since from the illustrious lecture delivered by Richard Feynman to the American Physical Society at California Institute of Technology in 1959. In his address entitled, “there is a plenty of room at the bottom”, he outlined the idea of building objects from the individual atoms through manipulation, and precise control of things on a small scale [5]. Later, the research in the field of

nanoscience gained a momentum with the discovery of the molecular beam epitaxy, the generation of nanoparticles, and the invention of the scanning tunneling microscope, application to optical systems, electronic, chemical, environmental engineering and medicine, leading into a robust and well-accepted scientific field (Fig. 2) [6].

The term ‘nano’ was originally derived from the Greek word, ‘nanos’ which means dwarf. Generally, nano is associated with the SI unit for length (meter) and one nanometer is one-billionth of the meter ($1\text{ nm}=10^{-9}\text{ m}$). Figure 3 shows some size scales from macro to atomic. A normal human eye at closest focus can just resolve $20\text{ }\mu\text{m}$. The molecular level contents of neurobiologically important systems like cell, neuronal junction, nucleic acid and other small molecules are shown schematically.

- *Nanoscience* was indeed elaborated as the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at larger scale [7].
- On the other hand, *nanotechnology* is defined as the design, characterization, production and application of structures, devices and systems by controlling shape and size at the nanometer scale in at least one dimension [8].
- Further, *nanomedicine* involves the application of nanotechnology to medicine which includes areas of healthcare, disease diagnosis and treatment. In general, nanomedicine is detailed as monitoring, repair, construction and control of human biological systems at the molecular level, using engineered molecular devices and nanostructures for molecular diagnostics, treatment, follow-up, and therapy of diseases [6].

The physical properties of the bulk particles remain same irrespective of their shape and size and in addition, the properties of these substances are governed by the conventional mechanics. On the other hand, as the size of the bulk materials is reduced and reaches from micro to the nanoscale, the properties of the materials significantly alter and are controlled by the dimensions of the particles. A minute change in the size of the particles consequently alters the physicochemical properties of the particles. Due to the reduction in size to the nanoscale, the conventional physical properties that govern the bulk materials are no more followed by the nanoparticles and quantum mechanics come into play. The miniaturization of the materials to the nanoscale causes

- Quantum or material confinement resulting in tunable optical properties
- An increase in surface area per volume i.e., increased ratio of the particles on the surface compared to the total core
- Innate fluorescent properties of certain nanostructures

- Behavior similar to metallic or versatile semiconductors systems
- Excellent conduction of heat and electricity
- Excellent stiffness of nonomaterials (high Young's modulus)
- The molecular scale structure enabling coating with a layer of material as thin as one molecule
- Particle dimensions smaller than cellular size resulting in reduction in the average therapeutic dose and availability of the particles at the specific receptor interface

The increased surface area of nanostructures makes them highly unstable and reactive and therefore, nanomaterials are difficult to maintain as individual particles because of their marked propensity to agglomerate, since agglomeration reduces the enormous surface area in relation to the volume of the nanomaterial, which is energetically unfavorable [2]. Appropriate pharmaceutical approaches are in constant search to overcome agglomeration.

Knowledge in nanoscience and nanotechnology is gradually pervading into almost all the arenas of science and technology, leading to great scientific advances. Moreover, these aspects are expected to enroot fundamental changes in the design and application of nano scale materials, devices, and systems in varied fields. Nanotechnology is of particular interest to neuroscience because molecular and signal processing occurs at the micro scale of neurons which have distinct nanoscale compartments including synapses, axons and dendrites [9]. In case of targeting the drugs to the CNS, most part of the larger particles are readily metabolized by the liver and therefore, reducing the size of these particles enhances their propensity to remain in the vascular system and pass through the BBB. In addition, the attachment of inert nanoparticles or coating with nanomaterials allows for the targeting of the drug of interest to site-specific areas to avoid severe and adverse side effects to surrounding tissues. Table 1 enlists the ideal properties nanoparticles should possess in CNS targeted therapeutics.

Multifunctional nanomaterials

Nanomaterials are specific structure, or combination of structures, designed to deliver the therapeutic agent intact, directly to the site, at a much lower dose. These materials are usually specific and in the range of molecular structures that can interact with neurons or protein structures inside cells. Typically, the nanomaterials are delivered into the cell through either endocytosis or pinocytosis, but in some cases the molecule will pass through the cell membrane and into the cell with very little disruption to the lipid bilayer. The nanomaterials include several types of particles varying in both physical properties and in chemical compositions.

The nanoparticles are usually defined as the materials possessing more than one single dimension and being in a size range of 1-100 nm. Unlike macro or micro particles, nanoparticles

show unique size-dependent variation in physical and chemical properties. Nanoparticles are broadly classified into organic and inorganic particles based on their composition and constituents used in their preparation (Fig. 4).

Liposomes

Liposomes are self-assembling colloid structures composed of lipid bilayer and are the frontrunners in drug delivery systems because of their amphiphilic nature and resemblance to biological membrane [10]. They contain an inner hydrophilic core entrapping the hydrophilic drugs and the outer hydrophobic core to deliver hydrophobic drugs. The physicochemical properties of liposomes enhance their specificity, show excellent penetration and diffusion properties and overall they are nontoxic, being biocompatible and biodegradable. The liposomes are also tailored with several polymers ('stealth' coating) to reduce recognition by the reticuloendothelial system, and thereby to prolong circulation and retention time.

Dendrimers

Dendrimers are synthetic polymers made up of a central core surrounded with series of branched architecture, determining the functional characteristics. They are quite attractive systems for drug delivery and imaging diagnosis because of their nanometer size range, ease of preparation and functionalization (prospects to attach desired chemical groups), extremely low polydispersity, regular and high degree of branching, multi-valency, globular architecture, well-defined molecular weight and their ability to display multiple copies of surface groups for biological recognition processes [2].

Fullerenes

Fullerenes (buckminsterfullerene) are considered to be the third allotropes of carbon following diamond and graphite and are made up of sixty symmetrically arranged carbon atoms (C₆₀). Fullerenes are widely used for medical research because of flexibility and suitable for drug delivery systems. Recently, fullerene derivatives have been used for neuroregeneration studies, and thus fullerenes possess potential uses as materials in novel medical devices targeting the brain [11].

Various functionalizations have been performed to increase hydrophilicity of fullerenes as well as to prepare new compounds with biological and pharmacological activity. Water-soluble derivatives of fullerene derivatives found to be unique class of nanomaterials with potent antioxidant properties. Ongoing studies in animal models of neurodegenerative disorders suggest that these novel antioxidants are potential neuroprotective agents against excitotoxic, apoptotic, and metabolic insults [2]. The neuroprotective effect of fullerenes has been attributed to their redox properties and high affinity toward free radicals, as C₆₀ is capable of being reversibly

reduced by up to 6 electrons. Moreover, the addition of as many as 34 methyl radicals to a C₆₀ sphere has been reported, leading C₆₀ to be characterized as a “radical sponge” [12].

Carbon nanotubes

Carbon nanotubes are among the astonishing objects that science sometimes discovers and which will likely revolutionize technological developments of the 21st century [2]. Carbon nanotubes (CNTs) are the fourth allotropes of carbon, made up of C₆₀ fullerenes which act as the building blocks, elongated and rolled up to form a cylindrical sheets. They can be either single- or multi-walled and have the potential to act as bio-persistent fibres. Because of their typical properties of high mechanical and thermal stability, high thermal and electrical conductivity, CNTs have aroused a promising interest in their applicability ranging from transistor chips to neuronal scaffolds. Following the discovery that CNTs can penetrate cell membranes, the application of both single- and multi-walled CNTs has been a key focus for development of novel nanovector systems [13]. On the other hand, CNTs represent a scaffold composed of small fibers or tubes that have diameters similar to those of innate neural processes such as dendrites. Recent investigations have started to address the issue of the effects of CNT scaffolds for neural growth. Carbon nanotubes have also turned out to be a new array to improve the performances of recording devices. Because of the efficient charge transfer between CNTs and surface-anchored molecules, CNTs show high sensitivity to changes in surrounding electrostatic environments [14].

Carbon nanospheres

Carbon nanospheres (CNSPs) derived from the hydrothermal treatment of glucose. CNSPs are intrinsically fluorescent materials which do not require any additional fluorescent tags and thus have potential benefits in imaging. Furthermore, CNSPs are readily dispersible in water without any surface modifications and could successfully deliver molecules targeting the nucleus. The concentration of the CNSPs was found to be more compared to other peripheral tissues [15].

Quantum dots

Quantum dots (QDs) are colloidal, spherical, fluorescent nano-sized crystals, made of nearly every semiconductor metal (e.g., CdS, CdSe, CdTe, ZnS, PbS) and possess unique optical and electrical properties. Generally, quantum dots consist of a semiconductor core, over coated by a shell (eg. ZnS) to improve optical properties, and a cap enabling improved solubility in aqueous buffers [2]. QDs have a fine tunable broad absorption spectrum, narrow emission spectra and moreover, possess brighter emission and good photostability. QDs have also been enabled water soluble synthetically. In addition, the high quantum yield of QDs is not diminished upon conjugation with biological molecules such as proteins, oligonucleids, small molecules, etc.,

used to direct binding of the QDs to areas of interest for biolabelling and biosensing. In contrast to antibody labeling, their small size (10-30 nm) indicates they are far less likely to interfere with normal function than, for example, antibodies or fluorescently tagged proteins. These properties of QDs are gaining widespread recognition and are rapidly applied to fluorescent labeling of cellular proteins, cell tracking and in vivo imaging [16].

Magnetic nanoparticles

Magnetic nanoparticles are spherical nanocrystals with a magnetic core (Fe^{2+} or Fe^{3+}) with a polymer or metal coating which can be functionalized. Their magnetic properties make them effective agents to label biomolecules in bioassays, gene and drug delivery, as contrast enhancing agents in magnetic resonance imaging (MRI) and also in diagnostics. Superparamagnetic inorganic oxides such as Fe_3O_4 are proving especially useful in tumor targeting and imaging in several biomedical applications.

Gold nanoparticles

Gold nanoparticles are metallic nanoparticles which can be prepared with various geometries, such as nanospheres, nanoshells, nanorods and nanocages. These particles show localized surface plasmon resonance properties. This is a phenomenon whereby light induces collective oscillations of conductive metal electrons at a resonance frequency which in turn, determines the absorbing and scattering properties of the particle, so that the particles can be selectively activated. These particles are used as excellent labels for biosensors. Gold nanoshells are layered, concentric sphere nanoparticles consisting of a dielectric silica core and a gold shell. By manipulating the size of the silica core and the thickness of the gold shell, the plasmon resonance response can be tuned on.

Some recent application of the nanoparticles for the diagnosis and/or therapy central nervous system related condition is depicted in table 2.

Nanoscience in its reign

Nanotechnology in imaging

Among the imaging methods, functional magnetic resonance imaging (fMRI) is extensively used in most of the clinics to monitor brain activity in several neuronal disorders. When a brain area is more active it consumes more oxygen and to meet this increased oxygen demand blood flow increases to this area based on haemodynamic changes resulting from neuronal activity. Oxygen is carried by haemoglobin in capillary red blood cells and the haemoglobin behaves as diamagnetic when oxygenated and paramagnetic when deoxygenated. The variation in magnetic properties leads to small differences in the magnetic resonance signal of blood. This difference in

the varied blood oxygenation in accordance to the neural activity is used to detect brain activity with MRI, the method being called as blood oxygenation level dependent (BOLD) imaging.

In spite of this complex and informative assemblage of fMRI, the low signal-to-noise ratio could not provide an activated brain area precisely and the signal would not be efficiently analyzed from the intricate neuronal networks. Several studies are being taken up to improve the signal in neuronal imaging and especially in MRI. The introduction of nanotools and adjunctive alteration of the existing devices with the nanoparticles resulted in prominent benefits to overlook the limitations with the conventional devices. Ultra-small super-paramagnetic iron oxide nanoparticles coated with covalently bound bi-functional poly (ethylene glycol) polymers were engineered to enhance the brain functional responses. This nano-related modification of the iron technique has shown significant signals in event related (amphetamine-induced) changes in cerebral blood volume in different brain areas compared to the BOLD signals [17].

In addition, this *in vivo* imaging technique (MRI) is also based on the magnetic field strength and partial volume effects. Therefore, in the patients with certain physiological constraints, the spectral resolution remains constrained. On the other hand, the *in vitro* high resolution spectrometry includes extraction method where the sample undergoes several physical and chemical treatments and thus the histological characteristic of the sample is disturbed. In order to overcome these problems, a direct method, high resolution magic angle spinning proton MRI was developed. This device made up of a nano-probe needed only a small amount of sample, reduced deterioration of the sample, avoided partial volume effects and possessed high sensitivity [18].

Photoacoustic tomography (PAT) is a hybrid imaging technology that combines the ultrasonic and optical techniques, overcoming the resolution disadvantage of optical imaging caused by strong light scattering in tissue and the contrast and speckle disadvantage of pure ultrasonic imaging. PAT is a quiet promising imaging modality which is based on the principle of differential absorption of electromagnetic waves for different type of biological tissues. In PAT, a pulsed laser is usually used as a pumping source to irradiate an absorbing medium. Consequently, the thermal expansion of the instantaneously heated media induces acoustic waves that propagate through the medium with minimal distortion and can be detected at the surface of the medium by highly sensitive transducers [19]. Nanodevices including nanoshells and gold nanoparticles have found to possess considerable significance as new contrast-enhancing agents in PAT and other medical diagnosis. The plasmon resonance phenomenon of the gold nanoparticles is exploited in using these nanoparticles as imaging agents in near infrared region,

to which biological tissues are completely transmissible without damaging the tissues. The gold nanoshells were used in PAT imaging of rat brain with enhanced optical contrast [20].

Extensive research with the use of nanodevices was being performed to overcome the limitations regarding the sample preparation and labeling in electron microscopy. Alternatively, colloidal gold particles and quantum dots were conjugated to antibodies for labeling approaches which result in the superior and ultrastructural resolution. These nanoscale semiconductor particles, such as QDs, can also be visualized by electron microscopy and might therefore prove to be valuable tools in future immunolabeling studies.

Noncarriers for central nervous system drug delivery

The primary limiting factor for the delivery of the drugs to the brain is the presence of BBB which shields this tissue from the toxic and to the discontent of the researchers, the therapeutic agents. The tight extracellular junctions of the brain microvessel endothelial cells, absence of fenestrations and a diminished pinocytic activity and the direct communication between endothelial cells and astrocytes constitutes the structural integrity for the tight regulation of molecular transport from the blood into the brain interstitial fluid (Fig.1). Moreover, active efflux pumps and the degrading enzymes found in the endothelial cells prevent the xenobiotics from reaching the targeted level in the brain [21]. Even small molecules do not cross the BBB in pharmacologically significant amounts, unless the molecule is lipophilic, unionized at the physiological pH and has a low molecular weight (<400 Da) [22]. Novel strategies with nanotechnological approaches are aimed to make the transit easier for these agents and also to bypass the phagocytic and efflux mechanisms hindering the possibility in reaching therapeutic concentrations in brain. Nanomedicine has established new frontiers like improved biocompatibility, targeted delivery of the payload, smaller size, and more sophisticated electronics to neuro-targeting with enhanced therapeutic potential.

The nanoparticles used in the drug delivery systems usually are around 200 nm and the drugs or other agents may be dissolved into the nanoparticles, entrapped, encapsulated and/or adsorbed or attached. These systems are rapid and attractive because the methods of preparation are generally simple and easy to scale-up. The nanoparticles used in pharmaceutical drug targeting are generally but not necessarily composed of polymers which may or not be biodegradable. The transcytosis of nanoparticles at the BBB is dependent upon their size, surface morphology and ability to interact with the BBB endothelial cell surface. The current and the future therapeutic strategies are aimed at circumventing these problems in specific and effective delivery of the payload to the pathological tissues of the brain. The engineering of nanoparticles by overcoating with polymers, especially polysorbate 80 lead to sufficient adsorption of

apolipoproteins B and E from blood plasma onto the nanoparticle surface. These particles which mimic low density lipoproteins were found to interact with the low density lipoprotein receptors leading to their uptake by the endothelial cells of BBB [2].

Nanoprocessing can breathe new life into old drugs by reducing undesirable effects through efficient delivery of payload and increasing the bioavailability. Liposomes are, perhaps, the earliest type of nanomaterial developed for drug delivery. Several varieties of liposomes used to transport drugs over the BBB include small unilamellar vesicles, multilamellar vesicles, polyethylene glycol (PEG) coated liposomes, PEGylated ligand/antibody bearing immunoliposomes and stimuli sensitive liposomes.

Nanoparticles uptake by the innate phagocytic system plays an important role in their failure to reach the CNS in appreciable quantity. The coating of nanoparticles such as liposomes with hydrophilic polymers such as PEG, poly(alkylcyanoacrylates), poloxamines, poloxamers and polysaccharides have been proposed as an interesting alternative that can reduce opsonization and prolong circulation time and thus enhance targeted brain delivery [23]. This opsonization involves the interaction of colloidal particles with plasma proteins, especially with immunoglobulins, albumin, fibronectin, etc. Therefore, liposomes or other nanoparticles that present hydrophobic surface properties and/or efficiently coated with plasma components and rapidly removed from the circulation, since the macrophages of the liver and the spleen own their specific receptors for these opsonins. However, the same colloidal particles that are small and hydrophilic can escape, at least partially, from the opsonization process and consequently remain in the circulation for a relatively prolonged period of time [24]. The steric repulsion has been achieved by avoiding the deposition of plasma proteins on nanoparticles either through direct chemical link of PEG at the surface of the particles or adsorbing some surfactant molecules (copolymers of polyoxyethylene and polyoxypropylene). This altogether enhances particles' blood half-life and therefore, extravasations to nonreticuloendothelial system organs, including the brain, especially when brain pathologies occur together with an increase in the endothelial permeability [24,25]. Several mechanisms were hypothesized to explain the enhanced transit of coated nanoparticles for drug delivery to BBB which include the binding of nanoparticles to the inner endothelial lining of the brain capillaries providing a drug concentration gradient, and thus improving passive diffusion. On the other hand, brain endothelial cell uptake of nanoparticles was also put forward to occur through endocytosis or transcytosis [26]. Overall, the colloidal carriers such as liposomes and surface modified nanoparticles generally increase the specificity toward tissues, and improve the bioavailability of drugs by increasing their diffusion through biological membranes and/or to protect them against enzyme inactivation.

In one of studies conducted by the Afergan et al., the researchers have elegantly exploited the BBB transit of phagocytic immune cells by loading them with tailored liposomes containing serotonin, a neurotransmitter in central and peripheral nervous system in liposomes. These liposomes were in the range of 100-250 nm and are unusually not hydrophilic, do not have a neutral membrane and are not an ultra size. This results in the efficient uptake by phagocyte cells (monocytes and neutrophils), increased circulation period, and apart from undergoing rapidly clearance in liver and spleen, these phagocyte cells will transport the drug across the BBB with the drug then being released from the cells in the brain. Intravenous administration of these negatively-charged serotonin liposomes exhibited two times higher uptake than the free serotonin which otherwise does not transit BBB [27]. In addition, nanoparticles have also been used as a cargo for several proteins and peptides whose delivery to the brain is usually impeded. The nanoparticles conjugated with colloidal polymer of poly (butylcyanoacrylate) (PBCA) with desired peptide absorbed on to the surface and then coated with polysorbate 80 nanoparticles have been used as vectors for delivery of dalargin (an enkephalin analog with analgesic action). Oral administration of PBCA nanoparticulate delivery systems, double-coated with Tween 80 and poly ethylene glycol enhanced the brain delivery of dalargin that does not cross BBB by itself. This nanotreatment showed an enhanced anti-nociceptive effect of this peptide in animal studies [28].

Nucleic acid therapy is an emerging field in drug delivery research due to its potential for treating a variety of genetic disorders that have been incurable to date. This includes delivery of plasmid DNA or small RNA and nucleic acid carriers. Due to the low transfection efficiency of naked nucleic acid injections *in vitro* and *in vivo*, many nucleic acid delivery vehicles have thus been investigated. Though viral vectors have the merit of high transfectability, their potential safety risks as well as immunogenicity warrant the search for alternative non-viral vectors [29]. Further, the novel gene vectors are modified to tailor the drug delivery requirements including for transit of BBB. In one study, lysine modified chitosan magnetic nanospheres were prepared in which the average size of nanospheres was about 100 nm with narrower size distribution, good superparamagnetic property, perfect crystallinity and low toxicity. When DNA was conjugated with these modified magnetic nanospheres, the target DNA could successfully cross the BBB of rat [30]. Nanodevices are also being studied for their sustained action of the payload delivered to the targeted site in brain. Sustained release neuroactive compounds including neurotrophic factors were successfully encapsulated into poly (lactic acid-co-glycolic acid) (PLGA) polymer [31]. In addition, glial cell line-derived neurotrophic factor (GDNF) releasing microspheres were

also found show prolonged delivery of the neurotrophic factor for 2 months, allowing sprouting of the dopaminergic fibers in a partial rat model of Parkinson's disease [32].

Nanotechnology in neurotransmitter analysis

Brain is a focal point for several neurotransmitters/signaling molecules which monitor specific physiological functions of the body. The neurotransmitters are further defined as chemical messenger molecules located and released in the brain to allow an impulse to pass from one nerve cell to another. These neurotransmitters are broadly classified into excitatory and inhibitory types which upon release, either generate or inhibit a response by binding to their specific receptors. The most imperative signaling molecules of the brain implicated in several neuronal disorders include acetylcholine, dopamine, glutamate, γ -amino butyric acid, and glycine. For example, dopamine is one of the vital neurotransmitters that play a significant role in the functioning of central nervous, renal and hormonal systems as well as in drug addiction and Parkinson's disease [33].

In this context, estimation of the physiological levels of these neurotransmitters is crucial in exploring the disease mechanisms and in modification of the underlying pathologies by therapeutic agents. Starting from chromatographic techniques to advanced tandem mass spectroscopy, various sensitive and simple bio-analytical methods are being developed for the determination of these neurotransmitters precisely. Specificity is a major problem in bio-analytical methods due to the coexistence of several interfering biological compounds with similar nature. In case of dopamine estimation in biological samples, the interfering compounds such as ascorbic acid, whose oxidative potential is close to that of dopamine pose a great difficulty resulting in poor selectivity and reproducibility of the analytical method. Considerable efforts are being devoted to overcome such problems in most of the electrochemical and chromatographic techniques. A novel glassy carbon electrode modified with LaFeO₃ nanoparticles, approximately 22 nm in size was used in cyclic voltammetric determination of dopamine. This nano-enabled electrode exhibited excellent sensitivity, recovery, strong promoting effect and high stability toward the electrochemical oxidation of dopamine and in addition, the interference by ascorbic acid was eliminated efficiently [34].

Rapid strides have been achieved in the chromatographic research, considered to be an important tool in the biomedical analysis of neurotransmitters and other proteins. The considerable importance of the nanofield and its practice in the biomedical chromatography lead to the development of nano high performance liquid chromatography (nano-HPLC), which resulted in decrease of inner diameter (ID) of liquid chromatography column to allow for a smaller sample amount and to increase sensitivity and in addition a significant change in flow

rate. For example, normal HPLC includes columns with an ID of 4-5 mm and operated at a flow rate of 0.5-10 mL/min whereas nano-HPLC contains columns with an ID of 25-100 μ m with a flow rate of 24-4000 nL/min. This nano based HPLC associated with mass spectrometry (MS) or matrix-assisted laser desorption/ionisation (MALDI) is widely applied in neurotransmitter analysis and for the estimation of several vital biocomponents [35]. Some other important applications of nanotechnology in biomedical analysis are tabulated (Table 3).

Significant investigations focused on the determination of neurotransmitters with various modifications of the existing instrumentation and the use of nanotechnology to devise new and economical materials with high efficiency and convenience is highly commendable.

Efficiency of nanomedicine in pathophysiological conditions of brain

Brain tumors

Brain tumors remain the most devastating forms of human cancers with their relatively high morbidity, mortality and enormous cost of care. The primary brain tumors start in the brain, whereas secondary tumors spread to the brain from another site such as the breast or lung. The incidence of primary brain tumors is increasing, especially in the younger population as it represents the second cause of cancer death in adults less than 35 years of age. Brain tumors develop as a consequence of cellular genetic alterations that permit them to evade normal regulatory mechanisms and destruction by the immune system [36]. The World Health Organization (WHO) classification of tumors of the nervous system includes a grading scheme termed, 'malignancy scale' ranging across a wide variety of neoplasm [36]. The WHO classification is based on the cell origin and histological appearance and further, the grading system ranges from Grade I (least malignant) to Grade IV (most malignant), which signifies the rate of growth.

Among the brain tumors, half originate from glial cells and are thus classified as gliomas, and more than three quarters of all gliomas are astrocytomas [37]. Astrocytomas constitute a heterogeneous group of tumors that range from low grade to the most aggressive, glioblastoma multiforme, based on histopathological classification (from grade I to IV WHO).

Despite the recent improvements in the conventional surgical and adjuvant therapy for brain tumors, the multimodality approach currently used in the treatment of malignant brain tumors does not produce a desired progress in patient outcome [38]. The difficulty with treating brain tumors is the effective delivery of therapeutic or contrast agent to the tumor. Strategies aiming at surpassing the intricate BBB or which can enhance the specificity and retention time of the agents are the most sought in the advanced arena of brain tumor research. With this approach, researchers globally are fabricating novel delivery systems based on nanoplateforms which can

achieve the therapeutic efficacy or imaging contrast enhancement, by increasing the amount of therapeutic or contrast agents delivered to the specific site, and to minimize toxicity, or imaging background signal, by reducing systemic exposure.

A synthetic nano-low density lipoprotein (LDL) particle was developed as a drug delivery vehicle containing a lipophilic prodrug, paclitaxel oleate targeting the glioblastoma multiforme cells. This vehicle consisted of a synthetic peptide containing a lipid binding motif and the LDL receptor binding domain. Since LDL has been shown to be up regulated in glioblastoma multiforme, this approach enhanced the specificity of the drug delivery [39]. The favorable property of stealth coated colloidal nanoparticles (eg. Liposomes) having prolonged half-life in the blood compartment, allows them for selective access into pathological sites like tumors or inflamed regions with a leaky vasculature. The enhanced permeability and retention effects of polymeric liposomes and biocompatible dendrimers result in a higher drug accumulation in tumor tissues than in plasma and in other tissues. Recently, fabrication of doxorubicine loaded poly (alkylcyano) acrylate nanoparticles showed enhanced delivery of this antitumor agent across the BBB. The biodegradable polymeric nanoparticles were able to overcome the multidrug resistance which allows tumors to evade chemotherapy due to the overexpression of P-glycoprotein. The formation of an ion pair between the drug and the degradation product of coating polymer enabled the diffusion across tumor cell membrane [26]. Similarly, antitumor camptothecins encapsulated in polyester dendrimers showed an enhanced cellular uptake and an increase in drug retention within the human cancer cell lines [40].

Apart from polymeric nanoparticles, several varieties of magnetic nanoparticles including gold and iron oxide nanoparticles have also been evaluated as drug carriers for a variety of anticancer drugs. Kohler et al. demonstrated a sustained release of methotrexate in brain tumor cells delivered by iron oxide nanoparticles. In this study, the authors covalently attached methotrexate to nanoparticles through amide bonds to ensure stability of the drug conjugate under intravenous conditions. Through the use the covalent linkage, the controlled release of methotrexate to the cellular cytosol and subsequent cytotoxicity to these cancer cells was demonstrated [41]. Recently, highly functionalized and intrinsically fluorescent carbon nanospheres (Fig. 5) were fabricated which possess the ability to transit the blood-brain barrier and deliver drugs into cellular nucleus which regulate the gene expressions in tumors [15].

Despite the conventional chemotherapy and radiotherapy, thermotherapy using magnetic nanoparticles is a new approach of localized therapy, in which nanosized iron-oxide particles are directly introduced into a tumor location and subsequently heated in an alternating magnetic field. As the tumor cells are more sensitive to increase temperature than normal tissue cells,

tumor heating to temperatures between 41-45 °C induced destruction of pathologically degenerated cells [42]. Thermotherapy was found to possess impending value in successful treatment of tumors seated in deep body regions. On the other hand, positron emission topography in conjugation with the amino acid tracers allow a more precise estimation of tumour borders than magnetic resonance imaging and was found to be highly valuable for defining the target volume for thermotherapy [43].

Carbon nanotubes possess wonderful physical properties which make them promising tools for extensive purposes in medicine. Both single and multiple walled carbon nanotubes have vast applications due to their major role in gene and drug delivery and diagnosis of disease. Incubation of multi-walled carbon nanotubes with microglial cells did not result in proliferative or cytokine changes *in vitro* and were capable of transporting DNA and small interfering RNA molecules and exhibited an enhanced internalization capacity in phagocytic cells as compared to tumor cells. This study paved a way in using multi-walled carbon nanotubes as non-toxic and biodegradable nano-vehicles for targeted therapy in brain cancers [44].

The fluorescent properties of quantum dots make them excellent contrast agents for biomedical imaging due to their broad absorption spectra and narrow emission spectra, high sensitivity and stability. Quantum dots conjugated to tumor markers were widely used in effective diagnosis. Tumor markers are the specific or nonspecific proteins present both in the tumor vicinity and in the blood and urine of the cancer patients. Prolific exploitation of these markers resulted in a potential value for the timely diagnosis and treatment of the cancer. The epidermal growth factor receptor (EGFR), being one of the markers implicated in the development and progression of brain tumors was targeted with drug-coated quantum dots conjugated with the anti-EGFR antibodies. The study showed that these EGFR-targeted quantum dots enhanced optical imaging of brain tumors penetrate the glioma cells through anti-EGFR mediated endocytosis, suggesting effective targeting of the brain tumor cells by these remodeled nanoparticles [45]. Table 4 enlists certain applications of nanoparticles in preclinical and clinical studies in brain tumors.

Epilepsy

Epilepsy is a chronic CNS disorder, characterized by the recurrent episodes of spontaneous seizures due to neuronal hyperactivity in the brain and accompanied with or without loss of consciousness and has a number of subtypes [46,47]. A seizure is a behavioral manifestation of abnormal, disordered, spontaneous but synchronized, high frequency firing of neuron populations in the central nervous system [48]. Seizures or epileptic symptoms are can be viewed as resulting from an imbalance between excitatory and inhibitory processes on the brain.

proposed mechanisms for the generation and spread of seizure activity within the brain include abnormalities in the membrane properties of neurons, changes in the ionic microenvironment surrounding the neurons, decreased inhibitory neurotransmission which is primarily the γ -amino butyric acid (GABA) or enhanced excitatory neurotransmission which is primarily mediated by glutamate [49]. The conventional drug delivery systems available for the treatment of seizures include the oral delivery and in some cases intravenous administrations with currently available antiepileptic drugs. However, the existing therapies did not prove to be effective, and seizures persist in about 35% of patients with partial epilepsy [50]. The over expression of multi-drug drug transporters (eg. P-glycoprotein), naturally occurring for toxins and xenobiotics in the BBB was suggested to be responsible for this pharmacoresistance [51].

The blood-brain barrier and the blood-cerebrospinal fluid barrier form very effective barriers for the free distribution of many hydrophilic, polar drugs into the brain [52]. Therefore, several new strategies are being developed for the effective delivery and enhanced bioavailability of the antiepileptic drugs targeting the intricacies existing in the complex circuitry of the brain. In regard to the essential benefits possessed by the nanoparticles, a variety of nanodevices ranging from the liposomes to carbon nanotubes are tried to reduce *in vivo* degradation and systemic toxicity. Nanoparticles form solid, colloidal drug delivery system that consists of molecular (10-1000 nm) materials in which the active principle is dissolved, entrapped or encapsulated or onto which the active principle is adsorbed or attached. Brain targeted polymeric nanoparticles have been found to increase the therapeutic efficacy and reduce the toxicity for a large number of drugs. The synthesis of newer biodegradable polymers in nanoparticle formulations such as neuropeptide carriers has generated interest in the renewed approach to CNS drug delivery.

Several animal models are used to study the inherent antiepileptic activity for the molecular entities and on the other hand a vast number of drug delivery concepts including the ones using the nanoparticles are being explored for the administration of such drugs. Kindling is a chronic model of epilepsy where daily electrical and chemical stimulus induces a permanent change in the epileptogenic sensitivity of forebrain structures such that the initially ineffective stimuli become capable of evoking fully developed seizures [53]. Drugs reversing this kindling phenomenon are hypothesized to mitigate epileptogenesis. In one such model, thyrotropin-releasing hormone (TRH)-loaded copolymer microdisks implanted in a seizure focus was found to attenuate kindling development in terms of behavioral stage, afterdischarge and clonus duration. In addition, intranasal administration of TRH analogue and polylactide nanoparticles suppressed fully kindled seizures [54].

In order to overcome the pharmacoresistance due to the increased function of the drug efflux mechanisms such as P-glycoprotein and/or multidrug resistance-associated proteins of the BBB, the innate inhibitory mechanisms of the brain including the adenosinergic system has been extensively studied and tailored to suppress the seizure generation and spread in refractory cases. Scientists at the Legacy Research have shown that the nanofilm-coated silk fibroin scaffolds containing adenosine microspheres implanted in the hippocampal cleft of the rat brain released a predetermined amount of adenosine daily and thus suppressed the seizures effectively in the rat model of kindling epileptogenesis [55].

The microelectrodes used to measure single-neuron action potentials result in the damage to surrounding neuronal tissue that further results in the formation of non-conductive glial scar. To improve the biocompatibility of chronically implanted microelectrodes, Moxon et al., have hypothesized that manipulation of the surface properties of the microelectrodes from typical smooth surface to more complex and porous surface may result in the efficient recording systems. In addition, the porous structure was presumed to act as a drug-delivery reservoir to deliver bioactive agents in aiding the repair or survival of cells around the microelectrode. The authors have then tested the nano-porous silicon surface layer to increase the biocompatibility of conventional thin film ceramic-insulated multisite electrodes. The *in vitro* and *in vivo* tests have shown the neuronal growth on porous silicon surfaces and biocompatibility of these surfaces compared to smooth silicon surfaces [56].

Electrophysiological techniques including electroencephalography (EEG) is the most specific diagnostic method to define epileptic brain and to localize the epileptic zone. EEG shows specific characteristic features in different epileptic syndromes. Though more sophisticated imaging methods have arrived to image the structural damage, EEG is the conventional and most widely used tool to study the epileptic locus, seizure patterns and to delineate the disease course during the continued therapy. EEG recording are performed either through invasive (stereotaxically implanted electrodes) or non-invasive (surface electrodes). Invasive recording is done in patients in whom epileptic zone cannot be located with non-invasive diagnostic methods [57]. The unique properties of carbon nanofibres (CNFs) including biocompatibility, stability in physiological solutions, excellent electrochemical activity, improved neuronal interfacing with functionalization and direct neurochemical sensing have made them versatile systems for electrophysiological recordings. Vertically arranged CNFs coupled to high density microelectrode array was simultaneously demonstrated to stimulate and record electrophysiological activity from multiple hippocampal brain slice cultures [58]. Despite

the small size, the CNFs could stimulate the neuronal tissue and acquired the signals with very low noise (Fig. 6).

Apart from the conventional pharmacological and surgical treatments for epilepsy, a substantial number of patients either do not become seizure-free or they experience major adverse events. In this context, neurostimulation based approaches like vagus nerve stimulation and deep brain stimulation have gained considerable interest. Deep brain stimulation involves placement of a pacemaker in specific location of the brain which receives predetermined electrical impulses. The success of these methods which involve brain-machine interface depends on the electrodes that come into contact with the neural tissue. The techniques for deep brain stimulation have certain limitations, such as large size of electrodes (in comparison with small neuronal group stimulation), lack of feedback monitoring of brain electrical activity, and high electrical current needs. Carbon nanotubes have shown considerable efficiency in improving these electrophysiological methods wherein electrodes coated with carbon nanotubes could resolve these drawbacks and offer better prospects in monitoring neuronal activity. The conventional tungsten and stainless steel wire electrodes coated with carbon nanotubes exhibited both enhanced recording and electrical stimulation of neurons in both *in vitro* and *in vivo* studies by decreasing the electrode impedance and increasing charge transfer [59].

The imaging technique like magnetic resonance imaging (MRI) is widely used to outline structural abnormalities in several neuropathological conditions. Among the existing bioactive molecules, alpha methyl tryptophan (AMT) is a recently used surrogate marker to localize the epileptogenic region and study patterns of brain injury for instituting antiepileptogenic treatment and for several other purposes. Using nanotechnology AMT was covalently attached to magnetonanoparticles visible on MRI. These particles were found to cross the BBB and concentrate in epileptogenic tissues during the acute and chronic stages in an animal model of epilepsy and render these tissues visible on highly efficient MRI. The epileptogenicity of brain tissue demonstrating AMT bound magnetic nanoparticles uptake was also confirmed by the electrophysiological studies. Thus, this study proved that magnetic nanoparticle approach is potentially applicable to the use of bioactive molecules as ligands for imaging both normal and abnormal localized cerebral functions, with high structural resolution MRI [60].

Alzheimer's disease

Dementia is a neurological disorder characterized by progressive impairment of intellectual ability to carry out daily tasks. The most common form of dementia among older people is Alzheimer's disease (AD), affecting more than 37 million people worldwide [61]. AD is associated with shrinkage of the brain tissue with localized loss of neurons in the hippocampus

and basal forebrain which control thought, memory and language. Microscopically, aggregated synaptotoxic β -amyloid ($A\beta$) peptide, as well as neurofibrillary tangles composed of hyperphosphorylated tau protein, are considered to be the hallmarks of AD [61]. On the other, oxidative stress is also believed to be one of the important pathological factors of AD. The most widely employed pharmacological intervention is to promote cholinergic transmission, believed to be responsible for cognition and memory, and thus existing drugs for AD increase the availability of acetylcholine in central cholinergic pathways by inhibiting the enzyme, acetylcholinesterase [62]. More recent therapeutic strategies include preventing $A\beta$ formation, blocking its aggregation into plaques, lowering its soluble levels in the brain, and disassembling existing amyloid plaques in preventing the progression of AD.

BBB stands as a potential barrier for the passage of almost all the foreign particles and drug targets including those aiming at the mitigation of AD. Polymeric nanoparticles possess enhanced permeation and retention effects and the adeptly engineered polymeric carriers dose not induce allergic reactions. Selective targeting of drug loaded nanoparticles to cerebrovascular amyloid deposits is a challenging aspect and several studies are being carried out to increase the specific deliver of the payload to the brain. Moreover, interests in exploiting the nano-based materials for the development of *in vitro* models for studying the disease pathology of the AD is also in pace with the fabrication of new drug delivery systems.

In one of the recent studies, the researches have dispensed the smart nonovehicles to deliver either a diagnostic agent coated on the surface and/or a drug entrapped in its biocompatible polymeric core which is further coated with a biosensor that can facilitate in detection and specific targeting of pathological tissue containing amyloid deposits. The chitosan was used to generate 250 nm sized polymeric core which further additional benefits of nontoxic amino acid products following metabolism. The polyamine modified F(ab') portion of IgG4.1, an anti-amyloid antibody, coated as a biosensor on the nano-vector surface. The flow cytometry and confocal microscopy showed an enhanced transcytosis of these smart nano-vectors from the BBB endothelial cells and accumulation in brain tissues *in vivo* experiments (Agyare et al., 2008). On the other hand, gold colloidal nanoparticles with an average diameter of 20 nm, coated with $A\beta_{1-40}$ exhibited reversible aggregation process between pH 4 and pH 10. This has lead to the possible significance of metal surface engineered with nanoscale interfacial environment, geometrically mimicking the *in vivo* systems for delving into the disease mechanisms [63].

Parkinson's disease

Parkinson's disease (PD) is a chronic degenerative condition of the CNS that leads to severe difficulties with body motions. Typical symptoms, first appearing usually in adults of late middle age, include tremor, rigidity, slowed voluntary movements, unstable posture and shuffling gait [33]. The depletion of the dopaminergic neurons in the substantia nigra thereby decreases the dopamine levels in the corpus striatum that upsets the delicate balance between dopamine and acetylcholine, leading to symptoms of PD. The currently available pharmacological and non-pharmacological treatments are able to offer only symptomatic relief for patients. Available therapies aim to improve the functional ability of the patient under treatment; however they do not alter the progression of the neurodegenerative process. The need for newer and more effective agents is consequently receiving a great deal of attention and is subjected to extensive research.

Deep brain stimulation (DBS) is an alternative procedure used to destroy small regions of the brain and/or inhibit the transit of signals. This is one of the novel therapeutic interventions for surgical treatment for advanced PD due to the fact that this technique is potentially safer than other available options. In this method, a thin electrode implanted into the specific brain region prevents transmission of impulses for involuntary movements. However, a fruitful DBS procedure is also associated with adverse effects that include neurocognitive (worsening dyskinesia, parasthesias, speech and gait disturbances), and with side-effects created by spread of stimulation to surrounding structures, depending on the precise location of electrodes [64]. In addition, the large size of microelectrodes, lack of simultaneous monitoring of local brain electrical activity and neurotransmitters; the open-loop nature of the stimulation (i.e. not guided by brain electrochemical activity) add to the volume of limitations of this current technology. Developments in nanotechnology have indicated probable advantages for implantable carbon nanotubes and nanochips while addressing the drawbacks associated with this method [65]. Reducing the size of the monitoring and stimulating electrodes to the size of neural elements allows remarkable improvements in both monitoring and stimulation. In one study, carbon nanofiber nanoelectrodes were established to offer trimodal arrays which include monitoring electrical activity, monitoring neurotransmitter levels, and precise stimulation. Thus, DBS was maneuvered to follow the changes in brain electrical activity and/or neurotransmitter levels and thus result in a versatile closed-loop system [66]. Accordingly, these systems allow greater safety and precision for the delivery of impulses in the substantia nigra, and therefore reduce side effects regarding surgery and equipment malfunctions.

Implantable biomaterials are major risk factors for hospital-acquired infection. Biomaterials coated with silver oxide or silver alloy or impregnated silicone with nanoparticulate

silver metal have all been used in attempts to reduce infection in clinical set up. The electrodes used in DBS for the treatment of PD require adjustment every 4 weeks. Nanocoating the neural implant with nanosilver could keep the electrode clear of a foreign body reaction and would also ensure that both the strength and area of stimulation would only need to be adjusted annually [67].

Thin films of nanocrystalline inorganic elements grown on silicon substrates can serve as biosensors for the detection of the biological and pathological markers for the neurological disorders and serve as an analytical interface between living tissue and biomedical instruments. In one electrochemical test, TiN coated NiTi film exhibited better corrosion resistance as compared to uncoated NiTi film. These nanocrystalline films were grown on silicon substrate to improve the corrosion and mechanical properties. These silicon based nano electrodes were found to have potential utility in electrochemical sensing of dopamine, which has a critical clinical relevance in Parkinson's disease [68].

Huntington's disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease characterized by chorea (excessive, spontaneous, irregularly timed abrupt movements), loss of ability in controlling voluntary movements, cognitive impairment, dementia, and emotional regulation over a period of about 30 years [69]. HD is one of the inherited and progressive disorders in which the underlying mutation is caused by a cytosine-adenine-guanosine expansion in the first exon, within the coding sequence of the affected huntingtin gene [69]. Characteristic neuropathological features of HD are macroscopic atrophy of the caudate nucleus, presence of expanded polyQ containing proteins, neuron loss, and astrogliosis in the striatum, prone to the earliest degeneration in brain. Though the exact pathogenesis of this disease is not yet known, reduction in striatal inhibitory γ -aminobutyric acid (GABA) levels and alterations in benzodiazepine receptors, allosterically linked to the GABA_A receptor besides free radical generation and oxidative stress have been implicated [70].

One of the current challenges in clinical HD research is the identification of sensitive and reliable biomarkers to detect progressive neurodegeneration and neural dysfunction. The advent of the sophisticated and complex imaging techniques have established that clinical impairments and brain atrophy can be detected sooner, prior to receiving a clinical diagnosis. Therefore, efforts in developing functional neuroimaging approaches have gained momentum in HD research to provide potential, sensitive and reliable non-invasive tools to assess dynamic effects of specific brain regions. Nano-analytical techniques including nano-LC-MS/MS was applied to identify the proteins critical for the progression of HD and to study quantitative changes in these

protein expression in the striatum of transgenic HD mouse model. The application of nano-related technologies in adjunct to other translational technologies was found to be valuable in identifying proteins critical for pathological functions of HD [71]. Nanotechnology was efficiently used to model pathological and behavioral aspects of genetic brain diseases including HD and thus nanomaterials are being taken up as novel research tools for *in vivo* testing neurotherapies. Injection of organically modified silica nanoparticles into striata of animal brain was shown to be effective substitutes for non-viral gene carrier in designing models and for examining the pathological changes in HD [72].

Multiple sclerosis

Multiple sclerosis is a chronic autoimmune inflammatory disorder of the brain involving demyelination of neurons and thereby impairing the nerve conduction. Both genetic and environmental causes for multiple sclerosis have been suggested. Further, the lifetime incidence of multiple sclerosis is 0.1 % in the normal population and siblings of multiple sclerosis patients carry a lifetime risk of 3 % [73]. Pathological observations indicate scattered focal areas of demyelination and axonal degeneration in the brain. The disease is characterized by weakness, numbness and tingling in limbs, paralysis, double vision and sphincter disturbances leading to urinary urgency. Current treatments for multiple sclerosis include immunomodulatory or immunosuppressive agents.

Different imaging techniques such as MRI of brain or cervical cord are often helpful in demonstrating the presence of a several lesions in patients with multiple sclerosis. The current MRI marker for inflammation is gadoliniumdiethylene-triamine-penta aceticacid (Gd-DTPA), which visualizes BBB leakage, occurring as a result of inflammation (Grossman et al., 1988). There is a great demand for the new contrast agents and several laboratories are working in improving the existing imaging methods to study the spatial and temporal discrepancies between BBB leakage and cellular infiltration in inflammation due to multiple sclerosis. New contrast agents based on ultra-small superparamagnetic nanoparticles of iron oxide (USPIO) with improved signaling properties have recently been developed for clinical MRI [74]. Phagocytosis of USPIO by cells of the monocyte/macrophage system visualizes cellular infiltration in diseases with high macrophage activity [74]. Thus, imaging with these magnetic nanoparticles, may complement Gd-DTPA in visualizing cellular aspects of inflammation in multiple sclerosis. In one study, done by Vellinga et al., the pluriformity of inflammation including cell infiltration was visualized in multiple sclerosis lesions, using a SHU555C, novel USPIO particle having several attributes in comparison to the existing ones. These novel agents provided

complementary insight into the underlying pathology of multiple sclerosis by providing a clinical relevance as potential MRI markers for disease severity and possibly treatment efficacy [75].

Therapeutic approaches to promote neuroprotection are presumed to compensate the neuronal loss and ultimately prevent or delay neurological disability, would be of great benefit for multiple sclerotic patients. Among the nanoparticles, fullerenes are proved to be powerful antioxidants, reacting readily and at a high rate with free radicals, which are often the cause of cell damage or death in several neuronal disorders [76]. Recently, a novel water-soluble fullerene derivative attached to an NMDA receptor antagonist, which combines antioxidant and anti-excitotoxic properties, was found to block axonal damage and reduce disease progression in multiple sclerosis [77]. In both *in vitro* and *in vivo* studies the fullerene derivative reduced disease progression with reduced axonal loss and demyelination in the spinal cord and thus supplemented the beneficial effects of fullerenes in devastating neuronal disorders like multiple sclerosis.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a late-onset progressive motor neuron disease due to degeneration of spinal, bulbar and cortical neurons, responsible for controlling voluntary muscles. The other pathological factors for ALS include mutations in Cu/Zn superoxide dismutase type-1 (SOD1) resulting in defective protein aggregation, mitochondrial dysfunction, neuroinflammation, growth factor deficiency, and glutamate excitotoxicity [78]. All the muscles under voluntary control are affected in ALS and patients lose their strength and the ability to move their body and eventually when muscles in the diaphragm and chest wall fail, patients lose the ability to breathe without ventilatory support. The diagnosis of this disorder is still clinical and there is a pronounced delay between the onset of symptoms and diagnosis. No present available test can provide a definitive diagnosis of ALS, although the presence of upper and lower motor neuron symptoms in a single limb is suggested [78]. Instead, the diagnosis of ALS is primarily based on the symptoms and signs the physician observes in the patient and a series of tests to rule out other diseases. There is an imminent requisite for potential biomarkers that are sensitive to the progression of disease and thus enhance the diagnostic process resulting in new drug targets. Proteomic research and other biomedical analytical approaches are identifying biomarkers from analysis of the blood and cerebrospinal fluid, as well as from neuroimaging and neurophysiology studies [79].

Gold nanoparticles (AuNP) which possess special optical properties and the potential to be used as an ideal colorimetric sensor, was functionalized with SOD1 monomer to readily interact with SOD1 aggregates and was used to detect structural evolution mimicking growth

process of the protein aggregation in ALS. Since the aggregated structure of SOD1 in motor neurons is a key feature of the pathology of ALS, a simple and sensitive colorimetric assay employing the SOD1 protein conjugated on the surface of gold nanoparticles was effectively used for the detection of structural evolution of SOD1 aggregates (Fig. 7). This is based on highly sensitive changes in the local dielectric environment of SOD1-AuNP which further alters the surface plasmon resonance spectra, even in the presence of extremely small amount of SOD1 aggregate in the probe solution. The proposed sensor system was found to give quantitative information in tracking the structural evolution of SOD1 aggregates and provides the prospective potential for definitive diagnosis in ALS ([111]). On the other hand, utilization of nano-flow liquid chromatography coupled to several advanced imaging techniques was also shown to enable the discovery of biomarkers in ALS patents [80].

Schizophrenia

Schizophrenia or psychosis is one of the most severe mental illnesses suffered nearly by 2% of the world population [49]. Unlike the vast applications of the nanoparticles in several neuronal disorders, the role of nanomedicine in schizophrenic research is incipient confined to the formation of effective drug delivery strategies aimed to enhance the bioavailability since the current antipsychotic drugs undergo extensive metabolism in the gastrointestinal tract. This approach may also circumvent the peripheral side effects associated with the antipsychotics usually taken for long duration and moreover, the nanotechnology aims at the specific pharmacological action of the applied nanodevices. Extended-release polymeric nanoparticles containing risperidone, an antipsychotic drug were made using poly (epsilon-caprolactone) and poloxamers as polymeric stabilizers. In *in vivo* preclinical studies, these nanoengineered risperidone formulations showed a prolonged antipsychotic effect with reduced dose and moreover, with least extrapyramidal side effects usually observed with the conventional formulations [81].

Elan's nanocrystal technology enhanced the clinical performance of poorly water-soluble drugs by transforming them into nanometer-sized particles (Elan Corporation, Dublin, Ireland). This technology has been applied for an injectable formulation of paliperidone palmitate, a drug for schizophrenia in phase III clinical trial. This technology reduced the size of the drug and increased the bioavailability [82]. Thus nano-processing was shown to enable a drug to be more efficiently used by biological system in facilitating the pharmaceutical factors like solubility and absorption; and in addition, surface modification of the drug nanoparticles also facilitate their release at the target site.

Since the pathophysiological mechanisms involved in the psychosis are derived from the action of the pharmacological agents, nanotechnology should probably be made use of in developing early diagnostic methods and other visualizing contrast agents for effective evaluation of pharmacologic actions that has great potential for understanding neurotransmitter dynamics in schizophrenic brain. In addition, search for the appropriate biomarker in cerebrospinal fluid can provide important information to diagnose and monitor disease progression in schizophrenic patients. Proteomic and bio-analytical methods using label-free nano-liquid chromatography and mass spectroscopy are in constant development for the biomarker discovery and for accurate estimation of neurotransmitter changes in psychotic conditions [83]. In near future, this will result in the establishment of appropriate disease mechanisms and will generate timely remedies and avoid the schizophrenia associated suicides.

Cerebral ischemia

Cerebral ischemia is one of the leading causes of death through the world. The function of the normal brain is highly dependent upon adequate tissue oxygenation and in case of ischemia, defined as a severe reduction of blood flow, the oxygen and nutrition transportation of the brain is severely effected which ultimately leads to tissue hypoxia and cell death. Ischemia generally results from cardiac arrest or severe systemic hypotension, or due to cerebral vascular atherosclerosis. Glutamate excitotoxicity, oxidative stress through excessive production of reactive oxygen species, induction of a series of inflammatory events, resulting in the infiltration of circulating immune cells and activation of resident cells are the other pathological factors which cause ischemia-related brain injury and eventually neuronal death. Thus, development of effective therapeutic and diagnostic methods to probe the neurochemical processes can result in early medical intervention, which is of great relevance to neuroprotective therapeutics for the ischemic injury [84].

The impact of nanotechnology has also remodeled the therapeutics of cerebral ischemia as evident from several experiments which made use of the nanobases for efficient drug delivery. In one preclinical study, superoxide dismutase, a free radical scavenger was attached to polymeric nanoparticles to enhance the transit across BBB which otherwise possess poor permeability. As a consequence, the polymeric superoxide dismutase particles showed an effective reduction in the damaged brain tissue and prevented the neuronal degeneration [85]. Similarly, a brain impermeable, specific caspase inhibitor, which significantly reduces vulnerability to the neuronal cell death following cerebral ischemia, was also conjugated with the chitosan nanospheres coated with PEG and engineered with monoclonal antibodies. This novel,

brain targeted nanoparticulate drug delivery system displayed an impressive translocation across the BBB after intravenous administration in mice [25].

New imaging techniques enabling *in vivo* assessment of phagocytic activity are warranted for effective estimation of post-ischemic inflammatory responses and their modification by pharmacological agents. MRI has become a widely used method for real-time imaging; tracking of pathophysiological alterations and was proved to be an important tool for the investigation of prolific ischemic therapeutics targeted at neuroprotection and attenuation of inflammation. These MRI techniques that utilize cell-specific superparamagnetic iron oxide nanoparticles labeled with specific biological markers are being developed as contrast probes for prospective diagnosis and therapy of cerebral ischemia. Nontoxic nanoemulsions of perfluorocarbons resulted in an efficient visualization of the inflammatory processes by MRI in animal models of cerebral ischemia where the nanoemulsion served as effective contrast agent with specific localization to effected brain tissue [86].

Oxidative stress and neuroprotection

Oxidative stress is generally defined as an imbalance of oxidant and antioxidant mechanisms of the biological system [87]. As envisaged in the previous sections, oxidative stress and associated oxidative damage are mediators of cellular injury in many neurodegenerative disorders such as cerebral ischemia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington chorea. A promising neuroprotective strategy involves mitigating the oxidative stress, supposed to play a key role in these neuropathological conditions. The same oxygen which is required for cells, also promotes oxidative degradation to biological components through the formation of free radical species, often termed reactive oxygen species (ROS). The production of these catastrophic oxygen (O_2^-) along with nitrogen (NO) and hydroxyl ($\cdot OH$) free radicals in the biological system was proposed to result from xanthine oxidase, NADPH oxidase, cytochrome P-450 enzymes, D-amino acid oxidase, D-glucose oxidase, nitric oxide synthases, and myeloperoxidase among others [88]. The extreme reactivity of the free radicals with cellular components like proteins and nucleic acids (DNA, RNA) results in deranged functional activities of these cellular molecules, leading to cellular/neuronal degeneration. Apart from these enzyme systems, lipid peroxidation, transition metals such as copper and iron as well as electron leakage of the normal mitochondrial electron-transport system are also considered as the potent inducers of the neuronal damage. In order to counteract the oxidative systems, the body activates several innate defense mechanisms which include production of antioxidative enzymes like superoxide dismutase (SOD) (which converts O_2^- to H_2O_2), catalase, glutathione peroxidase, heme oxygenase, and thioredoxin family proteins.

Mitochondria were proposed to be the major source and the target of intracellular ROS wherein, excess ROS causes mitochondrial depolarization associated with enhanced mitochondrial permeability. This subsequently releases small pro-apoptotic molecules such as cytochrome c, leading to activation of caspase cascades and apoptosis. Thus, therapies targeted against ROS hold great promise for minimizing cellular injury in oxidative stress-mediated neurodegeneration [89]. The looming potential of the nanoparticles in reversing the oxidative stress is being recognized since the observation that C60 fullerene molecules themselves show powerful antioxidant property, because of their ability to associate with mitochondria and to quench ROS more efficiently than conventional antioxidants [12]. Chronic treatment of a synthetic carboxy fullerene SOD in transgenic mice reduced age-associated oxidative stress and mitochondrial free radical production, and significantly extended lifespan [90]. The functionalisation of the fullerenes to make them more hydrophilic resulted in the production of various C60 derivatives, including polyhydroxylated C60 (fullerol) and malonic acid derivatives (carboxyfullerene) with potent ability to quench ROS. In addition, administration of hydrated C60 fullerenes to chronic alcohol treated rats resulted in protection of their brain tissues from oxidative stress, prevented loss of astrocytes and significantly improved behavioral response and eliminated emotional deficits induced by chronic alcohol uptake [91]. On the other hand, pretreatment with fullerol showed free radical scavenging activity and protection against 1-methyl-4-phenylpyridinium (MPP^+)-induced acute Parkinson's disease model in human neuroblastoma cells. These polyhydroxylated fullerenes exhibited significant protective effects on MPP^+ -induced loss in cell viability, decreases in mitochondrial function, and increases in the levels of reactive oxygen species and oxidative damage to DNA and proteins [92]. Besides C60 fullerenes, several other nanoparticles were also tried for their antioxidant properties. GSH-based functional oligomers with various PEG and PEG diacrylate nanoparticles were synthesized and tested human brain neuroblastoma cell cultures where the GSH oligomers were effective in alleviating the oxidative stress in tumor cell lines challenged with H_2O_2 [93]. Mitochondrial dysfunction and suppression of mitochondrial electron transport complexes are involved in the pathogenesis of several neurodegenerative disorders and therefore, antioxidant therapy with mitochondrial targeting of antioxidant agents is expected to be more beneficial [94]. In the mitochondrial respiratory chain, besides acting as a proton pump, the complex I enzymes transports electrons from NADH to CoQ, thereby forming CoQH_2 which functions as an antioxidant, essential for the defensive system against oxidative stress in tissues. To this view, platinum nanoparticles having an activity similar to that of NADH and reducing CoQ were prepared and were shown to mimic the enzymatic functions of the complex I [87]. Thus these

platinum nanoparticles were indicated as the potential therapeutic agents for oxidative stress diseases with suppressed mitochondrial complex I such as PD. Nanoparticles composed of cerium and yttrium oxides (CeO_2 and Y_2O_3) were also shown to possess antioxidant property and thus inhibit the ROS production pathway [95].

Besides the free radical quenching property of the fullerenes, the illumination of C60 with visible or UV light fosters its transition to a long-lived triplet excited state and the subsequent energy transfer to molecular oxygen, yielding a highly reactive singlet oxygen ($^1\text{O}_2$), which along with other ROS react with a wide range of biological targets involved in cellular signaling and cell damage [89]. This dual property of C60 to either quench or generate cell-damaging ROS is still an enigma which is under constant scrutiny. Hence, chemical modification of fullerenes aimed to increase their water solubility and reduced toxicity is warranted for their effective use as antioxidant agents in neurodegenerative disorders.

Neurotrauma

Neurotrauma or traumatic brain injury continues to be a massive public health crisis, even with the availability of modern medicine in the 21st century. The most common etiology for the neurotrauma includes the road accidents. When a considerable head injury occurs, cerebral edema often occurs, which increases the relative volume of the brain. Apart from this, extensive loss of cerebral parenchyma and the formation of a cavity in the brain due to the primary destruction and/or secondary pathophysiological injuries such as hemorrhage, ischemia, and inflammation also crop up [96]. Current therapeutic strategies for neurotrauma aim chiefly at preventing the hemorrhage and the tissue loss with pharmacological agents. In recent years, nanotechnology has also penetrated into the neurotrauma research where modification of the primary damage and prevention the secondary brain injury (systemic hypotension, ischemia, elevated intracranial pressure or oxidative stress) has been in constant pursuit.

The first report on the use of nanotechnology for achieving complete hemostasis in mammals appeared from the work of Ellis-Behnke et al., where a self-assembling peptide such as arginine that established a nanofiber barrier was found to achieve complete hemostasis within 15 seconds. This nano-mediated haemostasis involved stemming of the blood flow and facilitation of the adjacent cells to repair the injured site, immediately when applied directly to a wound in the brain, spinal cord and other tissues. This nanotechnology has overcome the limitations with the conventional haemostatic approaches without the use of pressure, cauterization, vasoconstriction, coagulation, or cross-linked adhesives [97]. In another experiment, self-assembling peptide nanofiber scaffold (SAPNS) was observed to successfully lead to the reconstruction of the acutely injured brain tissue in an animal model of traumatic brain injury

[98]. SAPNS is a liquid made from nanoscale peptides, similar to the native extracellular matrix. It has the tendency to readily fill the cavities and become integrated with host tissue after self-assembling to a hydrogel and can provide a true three-dimensional environment for cell growth and migration to reconstruct the lost tissue in the injured brain (Figure 8).

Spinal cord injury

Spinal cord injury (SCI) is a severe clinical disorder that results in lifetime disability due to destruction of long ascending and descending axonal fiber tracts which further triggers inflammation, inducing neural cell death which aggravates spinal cord repair [99]. Neuronal tissue regeneration and neuroprotection with several peptide drugs including neurotrophic factors along with measures to impede inflammatory processes are the current strategies in treating spinal cord injury. The need for tissue repair has encouraged the development of nanoplatforms that can be used to fill the large cystic cavities resulting from the injury in the spinal cord. In one of the experiments, self-assembled nanofibre scaffolds were found to provide pleasant environment for neural progenitor cell survival, migration, and differentiation both *in vitro* and *in vivo* and moreover, the nanoscaffolds were found to integrate and bridge the injured spinal [100].

The unique properties including vigor, flexibility, inertness, electrical conductivity, and availability of chemical functionalization make single-walled and multi-walled CNTs versatile candidates for biological applications both as sensors and stimulators in neuronal tissue engineering and as substrates for neuronal growth such as in regeneration after spinal cord or brain injury. CNTs can be systematically maneuvered by altering their chemical properties by attaching different functional groups and in addition, altering the charge carried by functionalized CNTs allows them to control the branching pattern of neuronal processes. This approach using CNTs has several advantages over current growth substrates in clinical set up since CNTs offer morphology reminiscent of neuronal process, long-term durability, inertness, and desired functionalization [101]. In one of the recent studies, functionalization of the single-walled CNTs with 4-benzoic acid or 4-tert-butylphenyl functional groups resulted in a better platform for cell viability and moreover, electrical coupling of these CNTs along with neuronal cultures resulted in robust voltage-activated currents when electrically stimulated through transparent and conductive nanofilms [102]. On the other hand, magnetic nanoparticle-based vector systems are considered to be safe compared to viral vectors and these functionalizable imaging agents promote intrinsic neural regeneration in SCI. Taking into advantage the deranged integrity of the BBB in pathological conditions including SCI, the intravenous administration of

the magnetic nanoparticles significantly accumulated in lesion sites, these nanoparticles were found to be effective gene delivery vectors in SCI [103].

Numerous nanodevices have been explored for therapeutic purpose to carry a drug in a controlled release fashion from the site of administration into the therapeutic target in SCI. Intraspinal injection of GDNF (a tropic factor for spinal cord motoneurons) encapsulated in PLGA nanoparticles into the injured spinal cord continually released the tropic factor which in turn induced an increase in neuronal survival and improved locomotory function in SCI rats [104]. In case of strategies aiming to mitigate the inflammatory markers following spinal cord injury, controlled, nanoparticle-enabled methylprednisolone, a steroidal anti-inflammatory agent was delivered to the injured spinal cord in rats. The local sustained delivery of this therapeutic agent reduced lesion volume and improved behavioral outcomes [105].

Similar to the use of iron oxide nanoparticles for imaging in various neuronal disorders these particles are also employed in imaging the lesioned sites following injury to spinal cord. The *in vivo* migration of stem cells labeled with simple iron oxide nanoparticles and polycation-bound iron oxide superparamagnetic nanoparticles were studied in rat model of SCI to obtain better results with cell labeling [106].

Regenerative medicine

Regenerative or reparative medicine is the remodeling and regeneration of tissues *in vivo* for the replacement, repair or functional enhancement of tissues and organs. This is a burgeoning field in which the mechanisms to harness the body's own capacity to regenerate itself is being explored with astonishing results. Exciting applications of nanotechnology have been reported in regenerative medicine. In clinical scenario, regenerative medicine comprise the manipulation of stem cells by nanoparticles and/or nanostructured surfaces as well as tissue engineering to treat organs lost as a result of disease and trauma. In this purview, the effects of structure and properties of nanomaterials on the proliferation and differentiation of neuronal cells have become a new interdisciplinary frontier in regeneration medicine and material science.

The reconstruction and regeneration of the degenerated neuronal tissue following pathological insult is a formidable task and various nanomaterials are being used as platforms to examine and nurture neurons in order to replace the degenerating neurofibers. The adherence and viability of neural cells varies with varying surface roughness at the nano scale. Manipulation of the surface behavior with nanoparticle coating in the molecular level resulted in improved efficiency of the nanoplatforms in gaining a base for neural growth in regenerative medicine. The use of atomic force microscopy and confocal laser scanning microscopy and other imaging techniques aided the observation of surface behavior by the nanoparticle treatment. The silicon

substrates were found to have a nano-range roughness achieved by chemical etching. The neurons cultured over the silicon base form dense neural networks with enhanced connectivity. Moreover, porous structures existed among the neuronal cells and the silicon substrates played were essential in the neuronal adhesion and neurite outgrowth on the inert silicon wafers [107].

Recent advances in nanotechnology have stimulated a renewed interest in the fabrication of novel neural biomaterials for example, carbon nanotubes that could effectively be used for the reestablishment of intricate and innate connections between neurons. The carbon nanotubes possess an enormous importance in tissue engineering as material for improved tracking of cells, sensing of microenvironments, delivering of transfection agents, and scaffolding for incorporating with the host's body. These versatile uses of the carbon nanotubes make them one of the most efficient scaffolds for neuronal outgrowths. Advanced techniques like lithography and chemical vapor deposition resulted in ganglionic projections adhering and assembling on the carbon nanotube templates and thus formed interconnected networks with pre-designed geometry and graph connectivity [108]. Multi-walled carbon nanotubes (MWCNTs) formed a scaffold to promote neuronal adhesion and survival (Fig. 9). The surface structure, composed of the films of MWCNT allowed neuron adhesion and moreover, the dendrites and axons were found to extend across MWCNTs, glia cells, and glass [109].

Future prospects of nanomedicine

This review primarily covered CNS targeted nanomedicines, designed for the improved drug delivery, imaging, electrophysiological and bio-analysis of neurotransmitters implicated in several neuronal disorders. Even though the plausible potential of the nanomedicine is extensively endorsed, the research into this little big science is still in its infancy. Being able to detect neurological disorders earlier or during the course of the disease offers better prospects for the future, both for effected individuals as well as the economy. The nanotechnological aspects are likely to attribute the existing therapies with pharmaceutical manipulations such as protection against degradation and elimination, and with improved access to target cells and tissues. The development of high-resolution imaging techniques for rapid, noninvasive monitoring of the *in vivo* metabolism and performance of targeted nanomedicines is receiving intense scrutiny, and will certainly facilitate the imaging-guided drug delivery to promote the optimal use of brain-targeted nanomedicines. The interdisciplinary research with nanotechnology and neuroscience for the effective drug delivery to brain and mitigation of neuronal maladies is widely expected to change the landscape of pharmaceutical and biotechnology industries in prospective years to come.

Nanoneuropharmacology

Pharmacology is a fascinating and multifaceted discipline which conceptualizes the study of drugs and their interaction with living organisms [49]. Neuropharmacology is the branch of pharmacology dealing particularly with the effects of drugs on the nervous system. Neuropharmacology is a field where the nanotechnology has to still permeate to garner better therapeutic prospects in devastating neuronal disorders. Additional areas of neuropharmacology likely to receive considerable attention from nanotechnology include:

- Nanotization of the neurosteroids, neuropeptides, and neurotrophic factors, fibroblast growth factor, glial-derived neurotrophic factor, etc., (which have low BBB penetrability) and to explore their pharmacological behavior to regenerate novel targets for therapeutics and diagnostic applications in stroke, Parkinson's, Alzheimer's, other neuronal disorders.
- Use of nanotechnology to modify the existing antipsychotic and antiparkinsonian drugs to enhance their specificity and reduce their potential adverse effects.
- Bringing the herbal extracts into the nano-purview will certainly alter the poor bioavailability characters of several potent natural neuropharmacological agents.
- Pharmacokinetic and pharmacodynamic modeling of the nanoparticulate drugs to reach the exact mechanism of action of the nanotized drug moieties in relation to their transit in the biological system
- Development of the nanoparticulated drugs for enrooting the P-glycoprotein mediated drug efflux which will be of potential benefit in overcoming pharmacoresistance in several neuronal disorders like epilepsy
- Designing for visualization of the drug delivery sites by combining therapeutic agents with imaging modalities which can specifically target the neuropathological location and deliver the payload.
- The design of systems that are able to respond to external stimuli, such as, hyperthermia, ultrasound, light and magnetic fields, and that can be triggered to release their contents or modify the neuronal vicinity as desired.
- The development of nanosystems which are able to co-deliver multiple pharmacological agents for combination therapy in neuronal disorders, such as the release of the dopa-decarboxylase inhibitors initially which prevents the metabolism of dopa, and subsequently release of the levodopa, a precursor of dopamine to replenish its loss in Parkinson's disease.
- Manipulation of the neuropharmacological agents with nanotechnology and recombinant DNA technology can enhance the transit through BBB and thus can foster excellent therapeutic strategies with increased specificity.

- More effective use of nano-HPLC to overcome the existing intricacies such as specificity and resolution in determining the neurotransmitter changes in various neurological disorders.
- Nanodevices should also substitute the conventional microdialysis probes to reduce surgical trauma and ischemia and thus to result in judicious estimation of extracellular neurochemistry in relation to disease status.
- Nanoplatforams for constant monitoring of the *in vivo* efficacy of neurotherapeutic and diagnostic agents.

The road lies ahead for the nanoneuropharmacology which should emanate as the functional field to deliver competent remedies to the patients suffering from neuronal and psychaiatric disorders. The researchers need to focus on this imminent promising field which curtails the need for surgical interventions in most delicate neruopathological conditions like seizures, brain tumors and Parkinson's disease.

Nanoneurotoxicology

Though the research and application of the nanoparticles for *in vivo* purpose is in full swing, it is yet to predict complete biodistribution, physiological and toxicological effects of these nanoparticles in general and the neurotoxicological profile in particular. Many areas of these nanoparticles are still unexplored, such as their potential adverse human health effects and furthermore, the number of studies taken up to address the possible toxic effects of multifunctional nanoparticles is still meager. It is still ambiguous whether nanoparticles are a boon or a bane for humans suffering. The exclusive physical and chemical properties of the nanoparticles including the small size, large surface area, high reactivity, and size-dependent variation of particle behavior could result in imminent adverse effects despite the beneficial properties foreseen elsewhere. Moreover, poor recognition of nanoparticles such as carbon nanotubes by innate phagocytic system is itself prohibitive towards controlling their biological action and thus ultimately results in both unpredictable and undesirable responses.

There are only a few studies which evaluated the benefits *vs* risks associated with exposure to nanoparticles. For example, quantum dots may seem harmless, but they could become unstable because of their sequestration in tissues and thus, long-term exposure *in vivo* may result in impaired cell structures and functions. Cadmium selenium quantum dots were shown to disturb intracellular calcium homeostasis, and persistently elevate intracellular calcium concentration in cultured hippocampal neurons, ultimately resulting in neuronal death [16]. On the other hand, addition of magnetic nanoparticles to immature components of glial cells was found to inhibit cell attachment and impeded subsequent cell growth. These observations have

proclaimed that nanoparticles have a greater adverse effect on young cells compared to mature ones [110]. Because of safety concerns associated with the exploitation of nanoparticles, additional research on the growing as well as the matured neurons is exceptionally warranted. Polymeric coating may certainly enhance the circulation time and target specificity of the nanoparticles such as liposomes. Although this long circulating nanoparticles increase the susceptibility of targeted brain tissue to the drug, they also prolong the exposure of other tissues and organs, which may cause systemic side effects and toxicity.

Despite the rapid progress of nanotechnology and its varied applications in neuroscience, the research relating to the potent unpleasant health effects due to prolonged exposure to nanomaterials at various concentration levels is still rudimentary. In particular, the behavior of nanoparticles inside the cells and especially in neurons is still an enigma, and the metabolic and immunological responses induced by these particles are still in debate. Regardless of the prospective benefits offered by the nanomedicine, the neuroscientists should foresee and weigh the neurotoxicological profile of nanoparticles for human use. Notwithstanding the potential of nanoscience in bringing incurable and devastating neuronal disorders under the umbrella of curable disorders, the scientists should critically evaluate the imminent toxicological data which otherwise may worsen the neurophysiology in long term.

In this context, nanoneurotoxicology should itself emerge as an effective field and take up the challenge to decipher the cellular and molecular events that regulate the possible accumulation of nanoparticles in neuronal tissues and to estimate the potent and latent neurotoxic effects of these nanoparticles on chronic use. Pharmaceutical guidelines for the formulation and utilization of therapies which include the use of nanoparticles or nanodevices should come into strict scrutiny to prevent the impending health care risks, these tiny but powerful particles would emulate.

Conclusion

The outstanding application of nanotechnology in CNS disorders has remodeled the domain of diagnosis and therapy and thus nanomedicine promises to bring a great future to the individuals with functional neuronal derangement. Though the research in nanomedicine is still in its infancy, the potential benefits carved out till now and the possible prospects in ensuing years will reform the focus of nano and neuroscientists and other health care professional to attain efficacious neuronal remedies. Most of the neuropharmacological agents including the herbal preparation need to be nanotized to derive favorable effects unmet with the existing therapies. On the other hand, considering that a great fraction of nanoparticles (metal and inorganic) in biomedical applications are non-biodegradable, the current research should focus on the

biodegradable nanoparticles and also tailor the inert nanoparticles in a manner to be detected and degraded by the biological system. Moreover, the long-term effects associated with the nanomedicine must be thoroughly assessed. Further, extensive and innovative research in basic and methodological aspects of nanomedicine should be accomplished to address the earlier stages of the neuronal disorders for their effective mitigation.

References

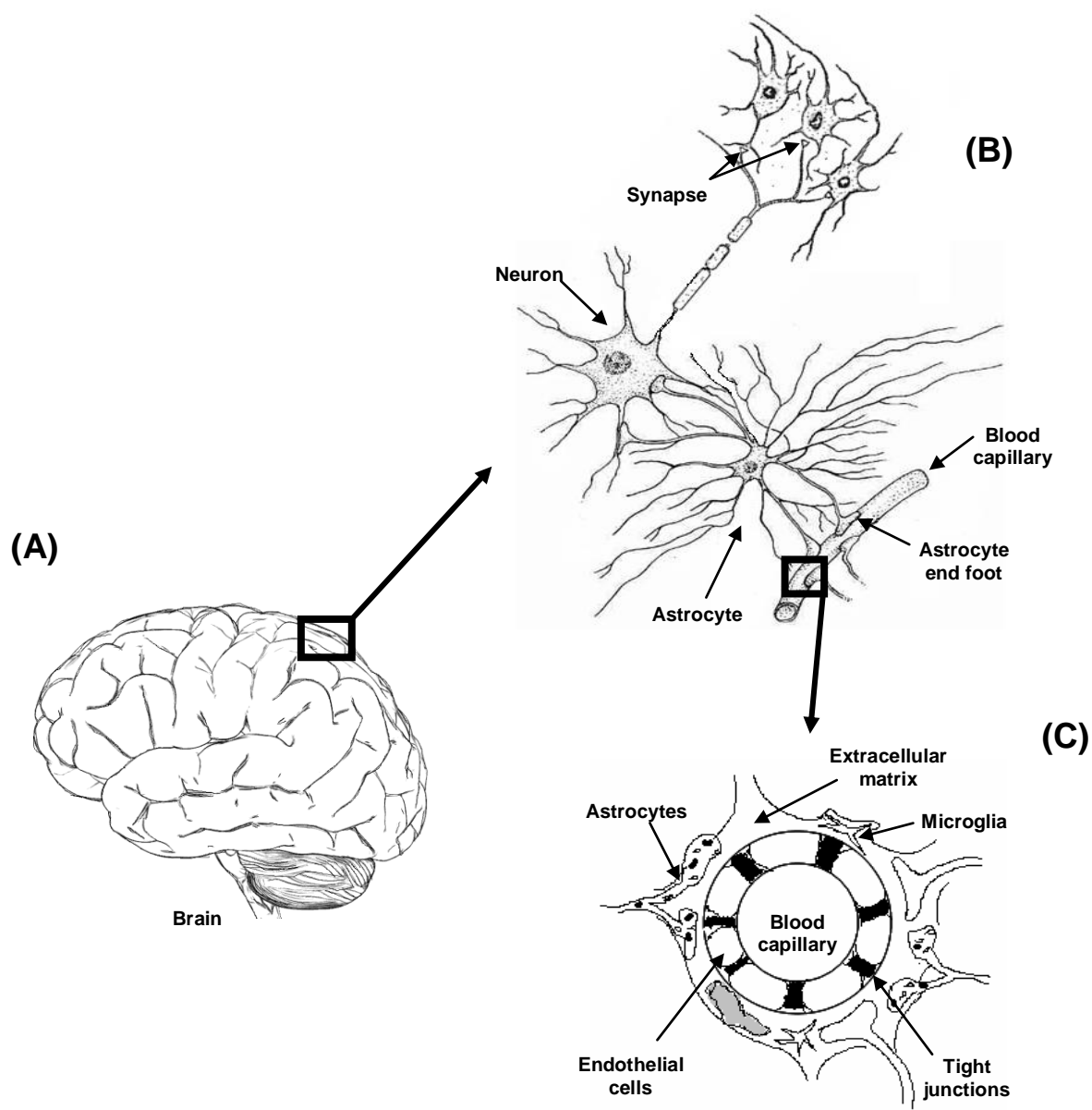


Figure 1. The human brain and its components. The inset from the whole brain shows the neuronal network with astrocytes and typical neuronal junctions (synapses). The astrocyte end foot onto the brain blood capillary is also depicted (B). The blood brain barrier with tight junctions between endothelial cells which prohibit the transit of drugs is shown (C).

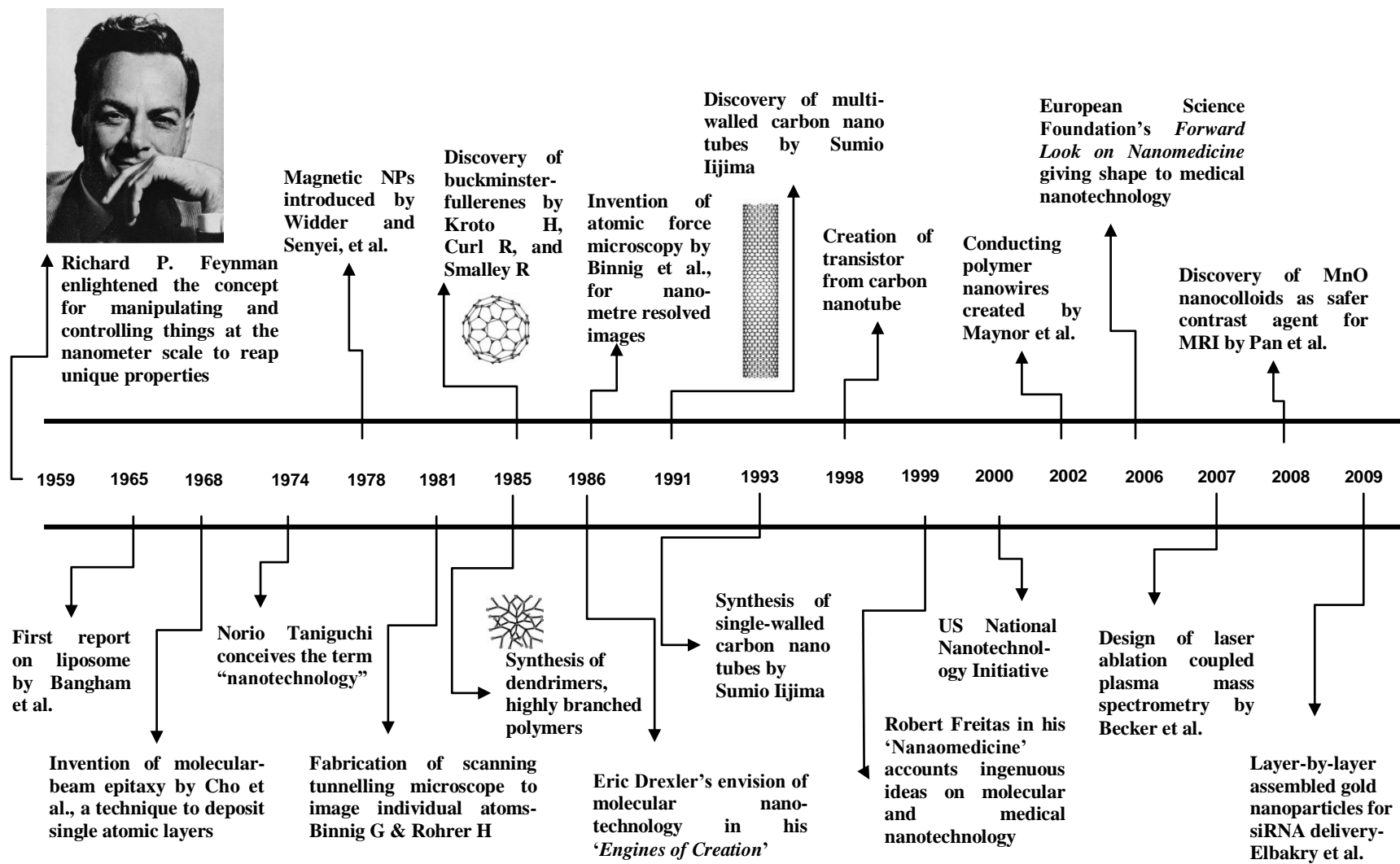


Figure 2. Fifty tremendous years of 'nano' concept: portray of historical development in nanoscience, nanotechnology and nanotools which ultimately nurtured nanomedicine. NPs, nanoparticles; siRNA, small interfering RNA.

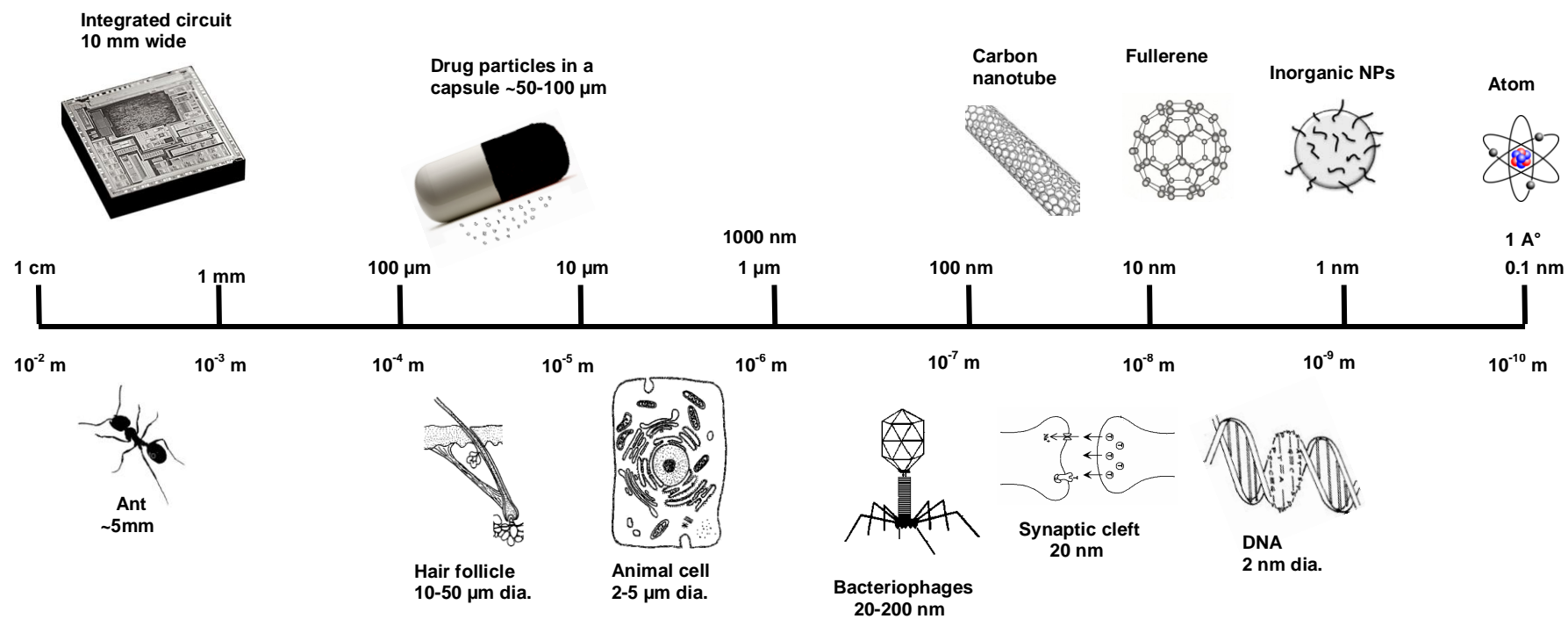


Figure 3. Some size scales from macro to atomic level. Top row above scale bar indicate the technical outcomes and the row below the scale bar indicate the naturally occurring biological entities.

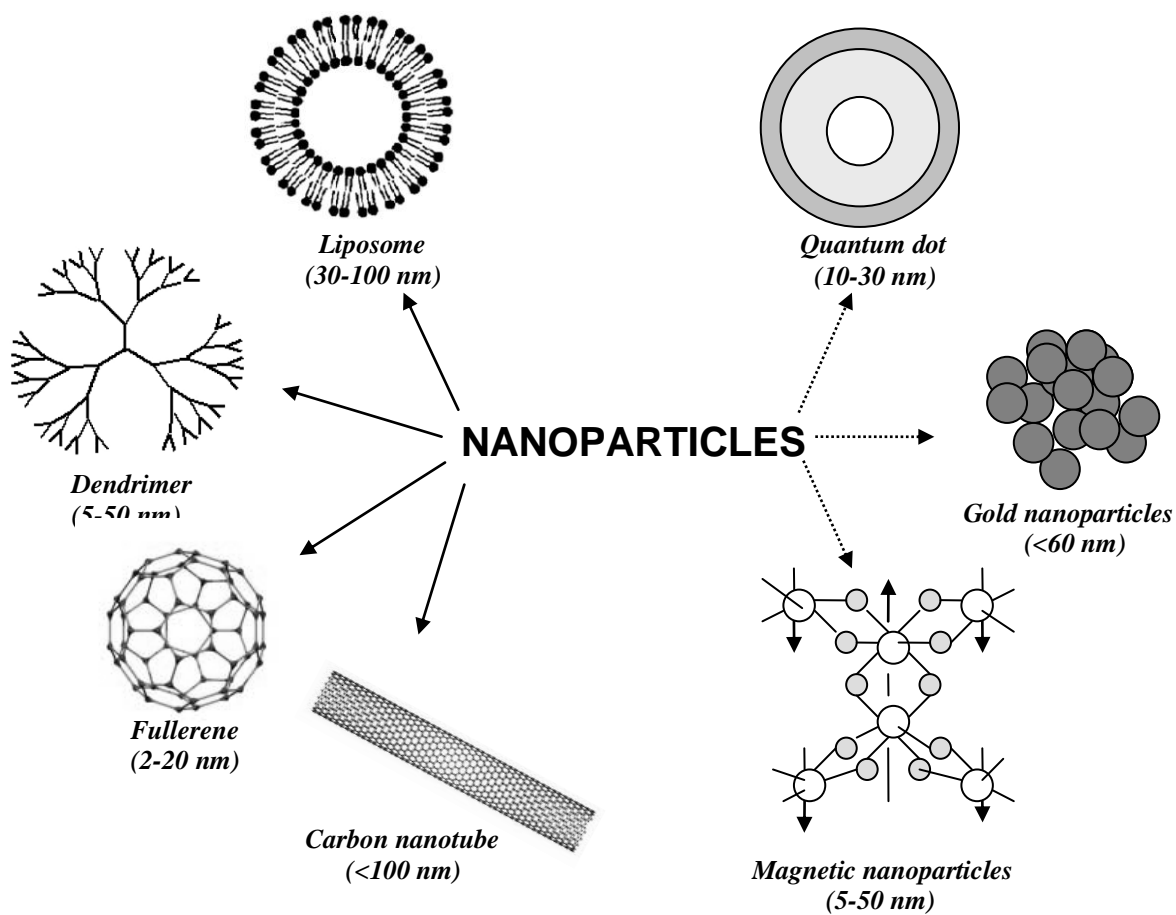


Figure 4. Pictorial depiction of nanoparticles. Straight line arrows indicate organic nanoparticles and dotted arrows indicate inorganic nanoparticles.

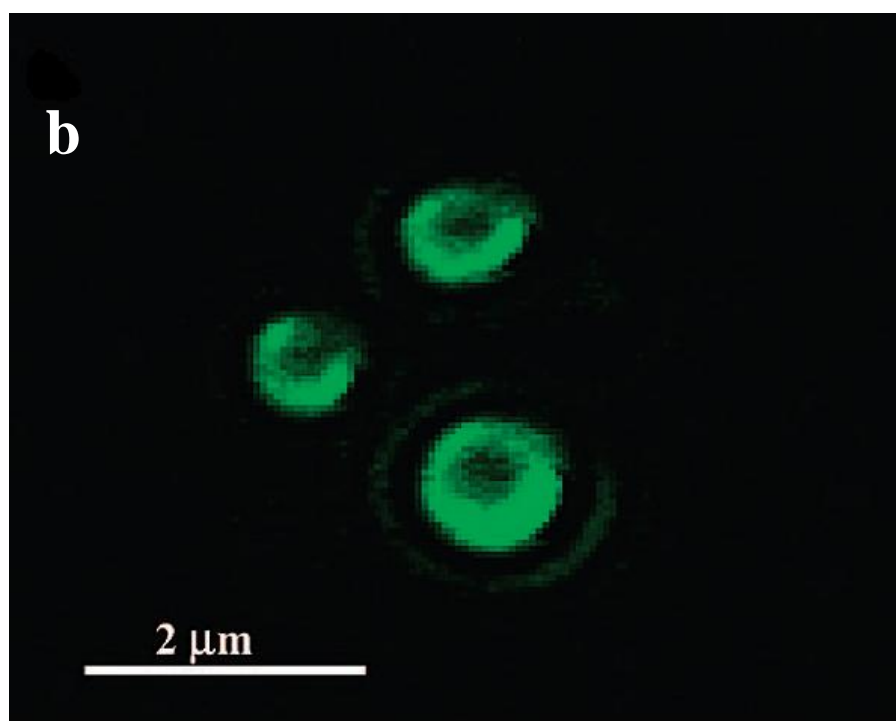
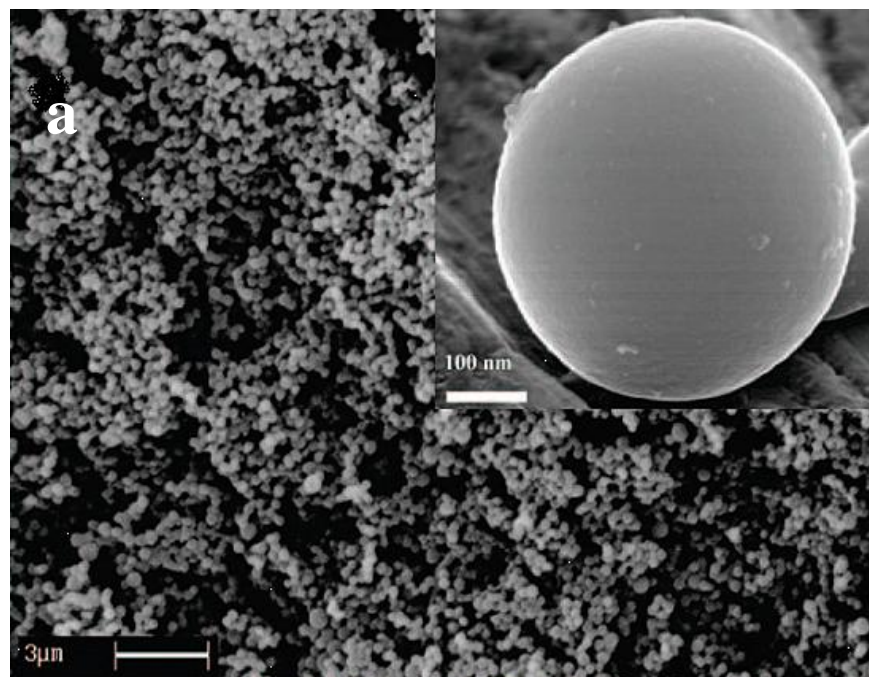


Figure 5. Intrinsically fluorescent carbon nanosphere. (a) Scanning electron microscopy image of the glucose-derived carbon spheres. Scale bar is 3 μm . Inset shows an isolated carbon sphere measuring about 400 nm. Inset scale bar is 100 nm. (b) Confocal laser scanning image of the carbon sphere obtained by using a drop of a water suspension containing carbon spheres. The fluorescence is obtained by exciting the spheres at 514 nm [15].

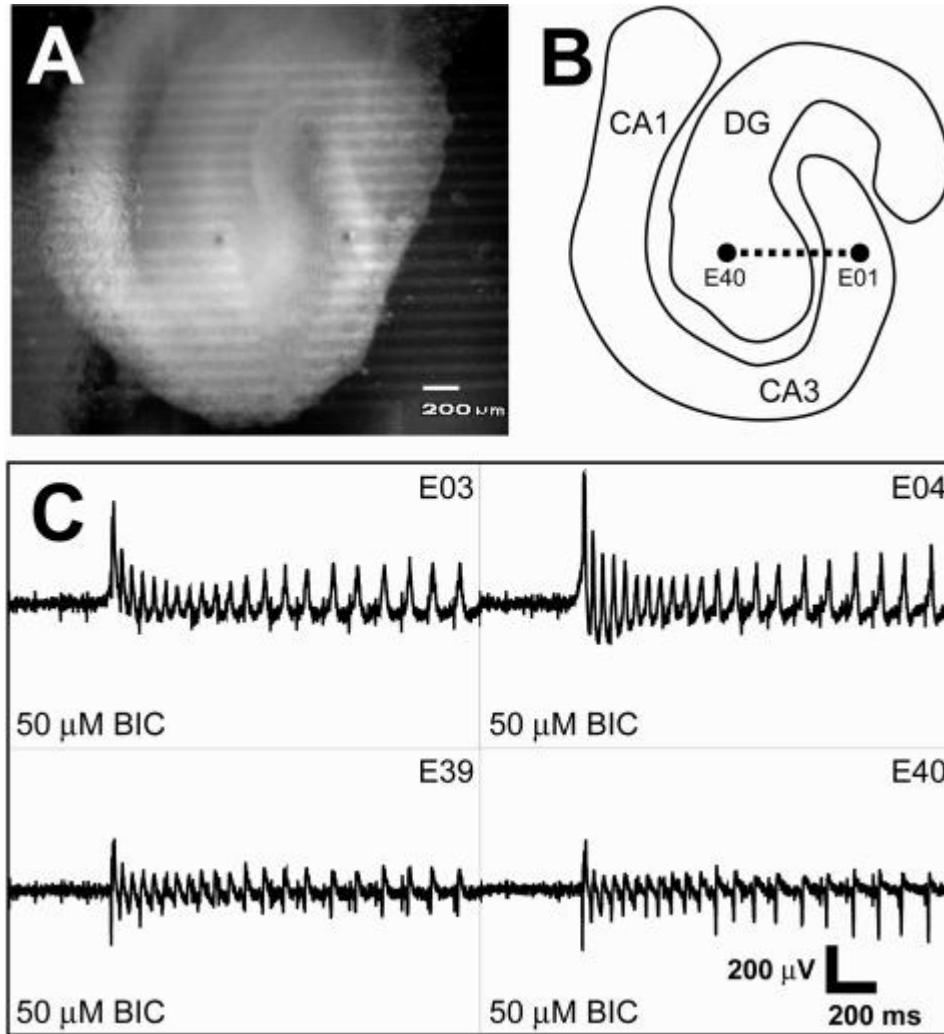


Figure 6. Bicuculline-induced epileptiform activity was recorded from hippocampal slices with the vertically aligned carbon nanofiber (VACNF) array chips. (A) A light micrograph of a hippocampal slice on a VACNF array chip. (B) A schematic figure of the hippocampal anatomy depicting the electrode recording locations. The electrode array crossed the hilus region from the dentate gyrus (DG) granule cell layer to the CA3 pyramidal layer. (C) Bicuculline-induced epileptiform activity as shown for four channels (electrodes 3, 4, 39, and 40) as indicated by continuous large oscillations with amplitudes up to 600 μV [58].

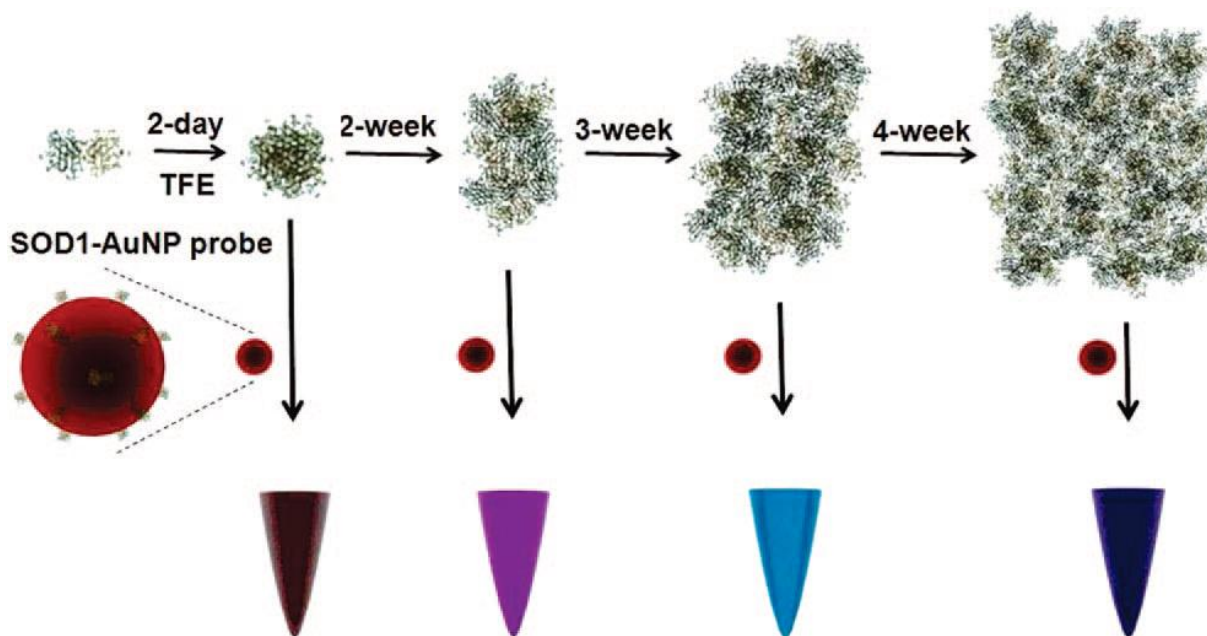


Figure 7. Schematic diagram showing the colorimetric detection system applied to the structural evolution of SOD1 aggregates. The samples of SOD1 aggregate were prepared by trifluoroethanol in weak acidic conditions (pH = 5.4) with an incubation time of ~4 weeks. After addition of the SOD1 aggregate samples into the prepared SOD1-AuNP solution, color changes in the probe solution (by shift of the surface plasmon peak) was observed by the naked eye and measured by UV-VIS spectroscopy [111].

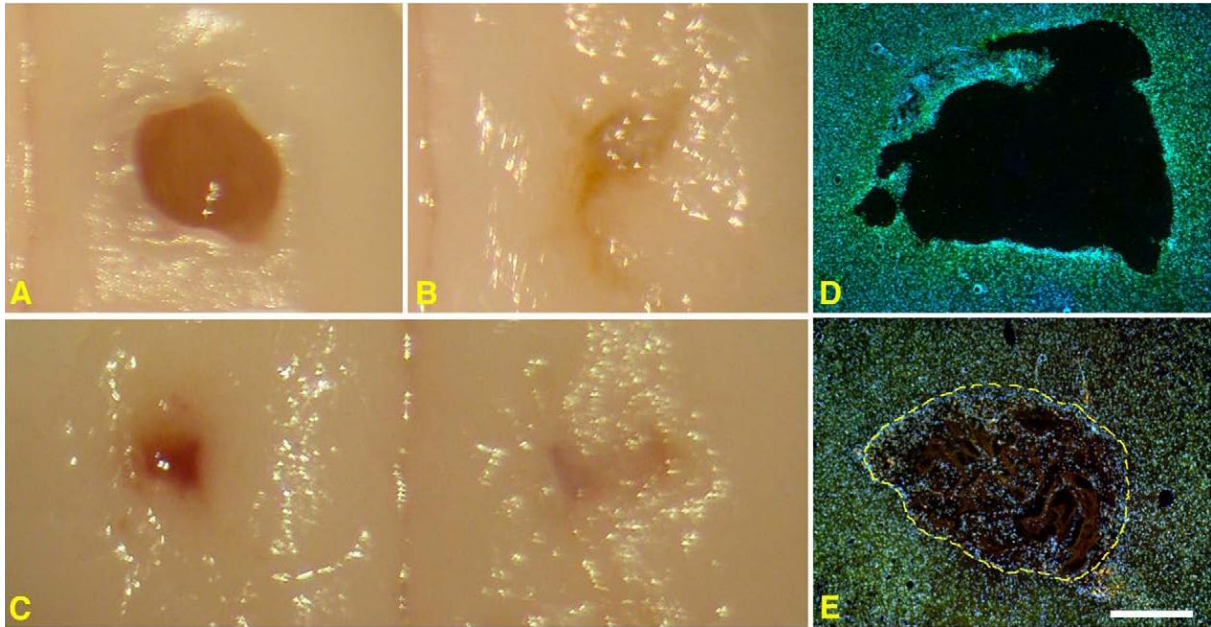


Figure 8. Lesion sites produced in an animal model of traumatic brain injury induced with unilateral cutting. The figure shows the lesions in different treatments, 6 weeks after surgery. (A-C) surface of the injured brain. (A) A moderate-sized cavity in the saline-treated brain with unilateral injury. (B) The wound in the self-assembling peptide nanofiber scaffold (SAPNS)-treated brain with unilateral injury has closed. (C) A sample of bilaterally injured brain with a small cavity in the saline-treated left hemisphere and SAPNS-filled lesion site in the right hemisphere. (D, E) Nissl and DAPI double staining showing lesion sites of saline (D) and SAPNS (E) treated brain. Panel E shows that the cavity formed by trauma was filled with SAPNS, which integrated very well with the host tissue with no obvious gaps. Scale bar: A-C = 1 mm; D, E = 500 μm [98].

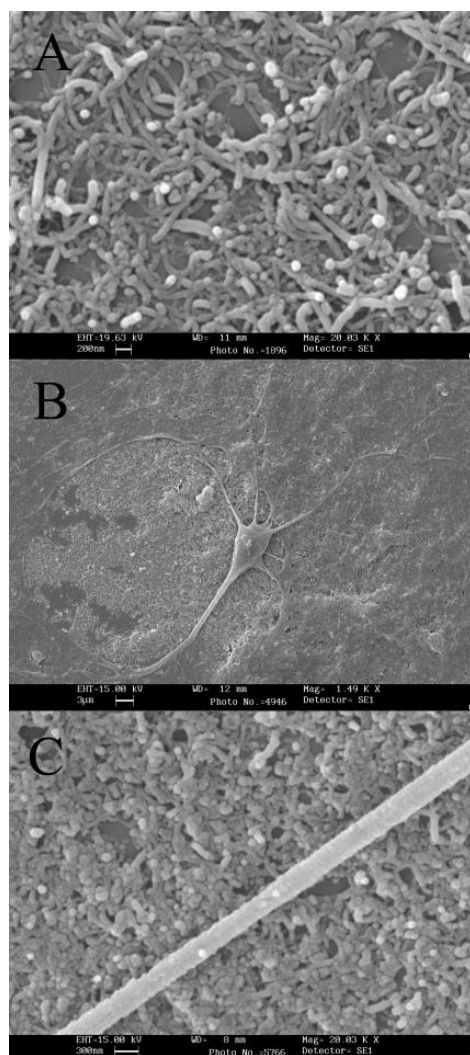


Figure 9. Purified multiwalled carbon nanotubes (MWCNTs) layered on glass are permissive substrates for neuron adhesion and survival. (A) Scanning electron microscopic monographs showing the retention on glass of MWCNT films after an 8-day test in culturing conditions. (B) Neonatal hippocampal neuron growing on dispersed MWCNTs after 8 days in culture. The relationship between dendrite and MWCNT is very clear in the image in (C), where a neurite is traveling in close contact to carbon nanotubes [109].

Table 1. Characteristics of ideal brain-targeted nanoparticles

-
- Ability to transit blood brain barrier with enhanced specificity
 - Should not be prone to phagocytosis by reticuloendothelial system
 - Ensure minimal drug degradation during transit to the target site
 - Should integrate with the neuronal network for predetermined period
 - Should be non-toxic and should not induce any neurodegeneration
 - Should not induce reactive oxygen species or free radical generation
 - Should not affect normal physiological function of the brain
 - Should not induce tolerance following chronic use
 - Should preserve the innate balance in neurotransmitter signaling
 - Should not induce seizures and effect normal cognition or memory
 - Should not affect surrounding neuronal tissue
 - Should prevent humoral response
 - Retain the drug at the target site for the desired period of time
 - Should attenuate drug clearance and increase circulation period
 - Facilitate cellular uptake and intracellular trafficking
 - Should possess improved range of imaging and with high resolution power
 - Should be biocompatible and biodegradable
-

Table 2. Applications of nonoparticles in the diagnosis or therapy of central nervous system related aspects

Nanoparticle component	Type	Purpose	Application	Nanotechnology	Outcome	Reference
Carbon nanotubes	MWCNTs	To evaluate internalization of MWCNTs in an intracranial glioma model	Brain tumor	MWCNTs were tagged with PKH26, a non-toxic, hydrophobic red fluorescent dye	Fluorescent labeled MWCNTs preferentially detected in tumor macrophages, and to a lesser extent in microglia. These findings highlight application of CNTs as a selective delivery vehicle into tumor macrophages in gliomas	[112]
		HPLC-ECD used to determine dopamine in microdialysate of rat striatum	Estimation of dopamine	MWCNT electrode chemically modified with carboxyl groups was used as the working electrode	Efficient electro-catalytic oxidation of dopamine with high sensitivity and stability and long life	[113]
	SWCNTs	Online electroanalytical method for continuous and simultaneous monitoring of glucose and lactate against the electrochemically active species endogenously existing in the cerebral systems in the rat brain microdialysate sample	Global cerebral ischemia/reperfusion	Electrochemical biosensors with plastic carbon film electrodes with engineered SWCNTs as the electrocatalyst	Excellent electrocatalytic activity of SWCNTs toward electrochemical oxidation with higher selectivity and tolerance against the variation of pH and O ₂ following disease condition	[84]

Contd...

SWCNTs		To investigate the capability of hybrid system with CNT as electrical interfaces between neurons and ion-sensitive field-effect transistor (ISFET), and analyze the electrical interactions and record the neuronal electro-physiological activity	To analyze extracellular neuronal electrical activity	Neuronal cell membrane (simulated by means of a silicon neuron) and an ad hoc modified ISFET which incorporates a bundle of individual CNTs arranged so that the neuronal cell membrane is in close contact with the nanotubes	The hybrid system response amplitude was larger than that recorded by the ISFET without CNTs. Moreover, CNTs affect the shape of the recorded signals and promote an increase in the efficacy of neuronal signal transmission.	[14]
		To increase the sensitivity, promote electron transfer, and reduce fouling (electrode adsorption) of serotonin	Fast-scan cyclic voltammetry for simultaneous determination of dopamine and serotonin <i>in vivo</i>	Carbon-fiber micro-electrodes modified with SWCNTs	Greater sensitivity and resistance to fouling	[114]
		To study effect of SWCNT graft copolymers on modulation of neuronal processes outgrowth	Regenerative medicine	Functionalization of SWNTs with poly-m-aminobenzene sulphonic acid and polyethylene glycol to enhance water solubility	In culturing medium, the functionalized water soluble SWCNTs increased the length of neuronal processes	[115]
		To transport CTPB, a membrane-impermeable molecule into nucleus which directly activates enzymes to regulate the transcription factor in tumors	Regulation of gene expression in (brain) tumors	Intrinsically fluorescent carbon nanospheres which can traverse the BBB and deliver drug into cellular nucleus	Effective drug delivery to nucleus, and uniform distribution of the fluorine signal.	[15]
Carbon nanospheres	-					
Carbon nanofiber	-	Deep brain stimulation	Parkinson's disease	Carbon nanofiber nanoelectrode array	Trimodal nanoelectrode arrays which result in monitoring electrical activity, monitoring neurotransmitter levels, and precise stimulation	[66]

Quantum dots	CdSe quantum dot	To determine effective diffusion coefficients of fluorescent probes and explicitly test whether a substance larger than 20 nm could diffuse through brain extracellular space (ECS) <i>in vivo</i>	Integrative optical imaging	Quantum dot conjugate [polyethylene glycol-coated quantum dots with a cadmium selenide core tuned to emit at 655 nm (QD655)]	QD655 measurably diffuses within rat neocortical ECS <i>in vivo</i> and the width of ECS is 38-64 nm, significantly larger than previous estimates based on electron microscopy, where 10- to 20-nm-wide clefts are typically observed	[116]
Magnetic nanoparticles	Iron oxide	To demonstrate the delivery of bone marrow precursor cells into the spinal cord via lumbar puncture (LP) technique	Spinal cord injury	Magnetic labeling of bone marrow cells with magnetic beads coated with a monoclonal antibody	Bone marrow cells labeled with magnetic nanoparticles delivered into the spinal cord via LP technique effectively migrated into the injured site in patients with chronic SCI.	[117]
Dendrimers	polyether-co polyester (PEPE) dendrimers	Evaluation of PEPE dendrimers as drug carriers for the treatment of gliomas	U87 MG and U343 MGa glioma cells	Methotrexate (an antitumor drug) - loaded dendrimers conjugated to d-glucosamine as the ligand for enhancing BBB permeability and tumor targeting	Enhanced endocytosis of glucosylated dendrimers by both tumor cell lines, increased potency of methotrexate resulting in reduction of tumor spheroid size	[118]
Gold nanoparticles	Planar gold nanoparticle array	Localized surface plasmon resonance to detect cellular activity from brain cells during their action potential-signalling at a single neuron level of spatial resolution	Detection of mammalian brain cell activity	Localized surface plasmon resonance of gold nanoparticles to intrinsically record neural cell activity at a single neuron level by optical means	Non-invasive technique with gold nanoparticles was shown to effectively record intrinsically neural cell activity at a single neuron level by optical means without additional chemical preparation or fluorolabeling	[119]

Table 3. Some bio-analytical methods using nanotechnology for neuroscience

Strategy	Nanotechnology	Outcome	Reference
Cyclic voltammetry	Nitrogen incorporated nanodiamond film as bio-sensing electrodes	Excellent electrochemical detection of dopamine	[120]
Differential pulse voltammetry	Nanoporous gold electrodes prepared by dealloying Ag from Au/Ag alloy with concentrated nitric acid	Substantial enhancement in electrochemical sensitivity for dopamine and ascorbic acid due to large surface area	[121]
Cyclic and square wave stripping voltammetry	Carbon, DNA-and mercury-immobilized carbon nanotube paste electrode	More sensitive at nano-range electrolyte detection with a speedy analytical time. Useful for pesticide assay in neuro-treated cell systems	[122]
Voltammetry	Determination of adenosine and dopamine using a single wall CNT modified glassy carbon electrode	Simultaneous estimation of adenosine and dopamine with good sensitivity and detection limits for physiological estimations in controlling Parkinson's disease	[123]
Liquid chromatography coupled to tandem mass spectrometry	Nano-scale liquid chromatography	Sensitive detection of 180 fmol of proteinase K-resistant prion protein per g brain as early as 24 h after inoculation	[124]
HPLC-ECD	Nano-Copper screen-printed electrode for the analysis of glucose concentration in brain microdialysate	Good linearity and reliable coefficient of variance for the estimation of glucose in brain samples extracted by microdialysis	[125]
Reagentless amperometric estimation	Functionalized MWCNTs with tin oxide nanoparticles as electrode	High sensitivity of the MWCNT-SnO ₂ modified enzyme electrode in monitoring of trace levels of uric acid in dialysate samples of rat striatum	[126]
NanoHPLC/MS/MS	Nanoelectrospray emitter packed with C18 reversed-phase particles, a lab on chip technique	Sensitive determination of histamine, a vital neurotransmitter in rat brain tissues with good linearity and detection limits in nanoscale	[35]
Electrochemical estimation of <i>in vivo</i> microdialysis samples	Enzyme electrode system based on electro-active neutral red-doped silica nanoparticles	Simultaneously monitoring of glucose, lactate, L-glutamate and hypoxanthine in diluted rat brain dialysate with high sensitivity, wide ranges of response, and without electroactive interferences	[127]
Cyclic voltammetry and differential pulse voltammetry	Electrochemical detection with nano crystalline manganese-doped lead dioxide film chemically modified electrode for liquid chromatography	Efficient estimation of the dopamine, norepinephrine, serotonin and their respective metabolites in microdialysate samples of rat brain	[128]

Table 4. Applications of nanoparticles in preclinical and clinical studies in brain tumors

Function	Nanotechnology	Active moiety	In vitro/ in vivo model	Outcome	Reference
Therapy	Polybutylcyanoacrylate nanoparticles coated with polysorbate 80	Gemcitabine	C6 glioma cells	Effective inhibition of the growth of C6 glioma cells <i>in vitro</i> and <i>in vivo</i> .	[34]
Imaging	Pegylated liposomal carrier encapsulating a contrast agent for contrast-enhanced MRI	Gadolinium	9 L glioma cells (rat glioma)	Development of a multifunctional long-circulating nanocarrier trackable by MRI	[129]
Therapy	Nano-low density lipoprotein (LDL) particle containing lipid binding motif and the LDL receptor binding domain of apolipoprotein B-100	Paclitaxel oleate	Glioblastoma multiforme cell lines	Selective drug delivery vehicle for targeting Glioblastoma multiforme tumors via the LDL receptor	[39]
Imaging	Nano-probe for acquisition high resolution magic angle spinning proton magnetic resonance spectroscopy	-	-	High sensitivity, direct method without physical or chemical treatment of sample. Fully automatic procedure, with decreased analysis time and potential costs of pathology MWCNTs did not result in proliferative or cytokine changes <i>in vitro</i> ; were capable of carrying DNA and siRNA and were internalized at higher levels in phagocytic cells as compared to tumor cells and therefore, could be used as a novel, non-toxic, and biodegradable nano-vehicles targeting brain tumors	[18]
Imaging	Multi-walled carbon nanotubes (MWCNTs) used to visualize <i>in vitro</i> ingestion, cytotoxicity, and loading capacity in microglia and glioma cells	-	BV2 microglia and GL261 glioma cells	These bioconjugated quantum dots were found to bind selectively (in less than 15 min) to brain tumor cells expressing EGFR. Highly specific binding depended on the expression level of EGFR on the cell membrane	[44]
Imaging and Therapy	Streptavidin-coated quantum dots conjugated to anti-epidermal growth factor receptor (EGFR) antibodies	Streptavidin	Human U87 and SKMG-3 glioblastoma cells (High EGFR expression)		[45]

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