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Nanotechnology Based Treatments for Neurological Disorders from Genetics Perspective

Genetik Bakış Açısıyla Nörolojik Hastalıklarda Nanoteknolojiye Dayalı Tedaviler

Nicholas S. Kurek [1], Sathees B. Chandra[1]

[1] Dept of Biological, Chemical and Physical Sciences, Roosevelt University, Chicago, USA

ABSTRACT

Nanotechology involves the application, analysis and manipulation of nanomaterials. These materials have unique and medically useful properties due to their nanoscale parameters. Nanotechnology based treatments and diagnostics might eventually bring great relief to people suffering from neurological disorders including autism spectrum disorders, Alzheimer's disease and Parkinson's disorders. A large variety of nonmaterials such as viruses, carbon nanotubes, gold and silica nanoparticles, nanoshells, quantum dots, genetic material and proteins as well as hordes of other forms of nanotechnology have been researched in order to determine their potential in enhancing disease treatments and diagnostics. Nanotechnology has shown countless applications and might eventually be used in every biotech/health industry. Nevertheless, many nanomaterials may pose some safety risks and whether their benefits overweigh the risk is still being debated. Once the proper ethical and safety protocols are established and enough research is completed, nanotechnology is expected to benefit the mankind enormously. In this article, we will discuss and analyze many ways in which, nanotechnology based treatments and diagnostics will be used to help people with neurological disorders through the methods that we currently have at our disposal.

Key words: Nanotechnology, nanomaterial, nanoparticle, carbon nanotube, nanocrystal, quantum dot, neurological disorders

ÖZET

Nanoteknoloji nanomateryallerin uygulama, analizi ve kullanımını içerir. Bu materyaller nano

ölçekteki parametrelerinden dolayı kendine özgü tıbbi açıdan yararlı özelliklere sahiptir. Nanoteknolojiye dayalı tedavi ve tanılar otizm spektrum bozuklukları, Alzheimer hastalığı ve Parkinson hastalığı gibi nörolojik bozuklukları olan hastalara en sonunda büyük rahatlık sağlayabilecektir. Virüsler, karbon nano tüpleri, altın ve silika nano parçaıcıkları, kuantum noktaları, genetik materyal, ve proteinler gibi çok farklı materyal olmayan nesneler ve diğer nanotteknoloji formları hastalıkların tanı ve tedavisini geliştirmekteki potansiyellerini tanımlayabilmek için araştırılmaktadır. Nanoteknoloji sayısız uygulama alanları mevcuttur ve bioteknoloji ve sağlık endüstrisinde kullanılması kaçınılmazdır. Bununla beraber, birçok nanomateryal bazı güvenlik riskleri taşırlar ve yarar zarar dengesi halen tartışılmaktadır. Uygun etik ve güvenlik protokolleri oluşturulduktan ve yeterli sayıda araştırma yapıldıktan sonra, nanoteknolojinin insan ırkını çok büyük katkılarının olması beklenmektedir. Bu yazıda, nanoteknolojiye dayalı tedavi ve tanıların nörolojik bozukluğu olan hastalara yardım için elimizde bulunan mevcut yöntemler aracılığıyla nasıl kullanılabileceği birçok yönden analiz edilerek tartışılmıştır.

Anahtar kelimeler: Nanoteknoloji, nanomateryal, nano parçacıklar, karbon nanotüpleri, nanokristal, kuantum noktaları, nörolojik bozukluklar

Introduction

Many neurological disorders cannot be cured or properly treated by conventional medicine[1,2] which can also induce devastating and sometimes permanent side effects like tardive dyskinesia and akathisia[3,4]. As our ability to use nanomaterials (NMs) in scientific applications continues to advance, nanotechnology will allow for better treatment and diagnostic techniques for neurological disorders. NMs have at least one dimension that is less than 100 nanometers and can consist of composites, metals, polymers and ceramics[5]. Examples of commonly used NMs include nanoparticles (NPs) like quantum dots (QDs) and nanoshells. Other NMs that have proven to be useful are liposomes, polymeric micelles, nanofibers, dendrimers and nanogels. NPs are solid colloidal particles which often consist of polymers that are biodegradable and insoluble[6] but can also be made up of metals like iron, gold and silver[7]. Crystalline NPs are often referred to as nanocrystals (NCs)[8,9]. QDs are fluorescent semiconducting NCs. Colloidal QDs are single crystals with diameters of only a few nanometers and can be controlled through the temperature, duration and ligand molecules used during their synthesis[10]. Nanoshells are NPs that contain di-electric cores and metallic shells[11]. It should also be noted that while viruses and proteins do not officially qualify as NMs, they are still very small scaled materials that are appropriate for this discussion.

Consequently we will also discuss applications involving viruses and proteins such as transduction and immunoaffinity assays.

Neurological disorders like autism spectrum disorders (ASDs)[12,13], epilepsy [14] and amyotrophic lateral sclerosis (ALS)[15,16,17] cause an enormous amount of pain, suffering and disability to a large percentage of the human population. ASDs follow a dominant inheritance pattern and are associated with other multifactorial neurobehavioral disorders such as Schizophrenia and Obsessive Compulsive Disorder[18]. Core symptoms of ASDs include stereotypical and repetitious behavior, delays in language development and impaired social interactions[19]. Seizures are often caused by abnormal astrocytic-neuronal signaling from excited glial cells to other neurons and astrocytes involving Ca^{2+} and Na^{+} [20] . Epilepsy can take many different forms and is the most common neurological condition found in children and adolescents. People afflicted with medical refractory epilepsy have a greater susceptibility to clinical complications that include injury and death[21]. ALS causes fatal neurodegeneration and the cause for it is not known. It is progressive and involves the selective death of lower and upper motor neurons, which leads to depression of respiration, paralysis and ultimately the patient's death within two to five years[22].

Nanotechnology related methods will drastically improve treatments and diagnostics for diseases like neurological disorders. In this article, we will discuss and analyze some fascinating and revolutionary nanotechnology based techniques currently at our disposal.

Gene Therapies for Neurological Disorders

Silica NPs modified by sodium iodide or sodium chloride have been used for the highly efficient transfer of DNA through the blood-brain barrier (BBB) and into brain cells along with many other cell types[23]. Organically modified silica (ORMOSIL) NPs have been effectively used as a non-viral gene transporter in the modeling of expanded polyQ peptide brain pathologies like Huntington's disease (HD) in mice. The lateral ventricles or striatums of mouse brains were injected with ORMOSIL NPs complexed with plasmids that express Hemaglutinin-tagged polypeptides containing either extended 127-glutamine repeats or 20 glutamine repeats. Mice that were transfected using ORMOSIL NPs exhibited both behavioral and biomolecular characteristics associated with neurodegenerative disorders. This demonstrates that nanotechnology can be a powerful tool for creating animal based disease models as well as the testing of single and multiple genetic therapies in vivo[24]. NPs have been successfully used

in the biolistic transfection of DNA and RNA into HEK293 cells and could also be applied in neuronal biolistic genetic transfections. This experiment showed that NPs are more effective than microparticles in the transfection of small cells[25] .

Self-assembling and non-toxic ternary complexes consisting of plasmid DNA, polyethylenimine of low molecular weight and targeting peptide have been used as a non-viral system to deliver genes into neuronal cells. These ternary complexes have specifically and efficiently mediated gene delivery into NGF receptor TrkA expressing cells where in vitro luciferase reporter gene expression has been improved by a factor of 1000 versus nontargeting control peptide complexes. In vivo dorsal root ganglia site transfection improved by a factor of 59 versus control ternary complexes[26]. An innovative recombinant baculovirus vector with a hybrid promoter was constructed through the fusion of the human cytomegalovirus (CMV) immediately early promoter enhancer and the human platelet-derived growth factor (PDGF) β-chain promoter, resulting in a 1000 time increase in neuronal transgene expression when compared to vectors containing only the PDGF promoter. NMs like this CMV-PDGF hybrid can be used in gene therapies to protect neurons from the degenerative effects of diseases like Parkinson's disease (PD), ALS and Alzheimer's disease (AD)[15] .

MicroRNA (miRNA) expression profiling has shown that miRNA dysregulation is a central feature of the pathological process for a wide range of neurological disorders, including AD, ASDs, HD, PD and schizophrenia. AAV9 and other viruses have been shown to cross the BBB, making them ideal materials for use in gene therapies that could be used to treat miRNA dysregulations in the central nervous system (CNS). Baculoviral vectors where adeno-associated virus inverted terminal repeats (ITRs) flank a neuron-specific promoter that is harbored by a luciferase reporter gene cassette have resulted in a significant increase in the duration of transgene expression[27]. A similar experiment involving the CERE-110 adeno-associated virus has been used to reliably and accurately deliver a controlled dose of nerve growth factor into the human Meynert nucleus basalis brain region in rats and monkeys. A phase 1 CERE-110 clinical trial was performed for the evaluation of its efficacy and safety as a treatment for AD[28].

Regenerative Therapies for Neurological Disorders

Carbon nanotubes (CNTs) have enormous potential for use in regenerative therapies due to their unique and useful properties. CNTs belong to the fullerene carbon allotrope class and consist of carbon atoms organized into hexagonal ring structures where some 7 or 5 membered rings provide structural curvature. CNT synthesis can be performed using chemical vapor deposition, laser ablation, high-pressure carbon monoxide and arc-burned graphite rod discharge[6]. Thermal conductivity values of isolated CNTs at room temperature can be as large as 6600 W/m·K, which is unusually high[29]. Single walled carbon nanotube (SWCNT) transport properties vary based on the CNT density when SWCNT networks are fabricated into thin film transistors. At lower CNT densities, these networks demonstrate electrical continuity while behaving like p-type semiconductors. At higher CNT densities, these networks exhibit much greater field-effect mobility and behave similar to narrow band gap semiconductors with high off-state currents[30] .

Nanofibers and CNTs have demonstrated promise as neuroregeneration scaffold implants that could be used to treat conditions like AD and PD along with injury induced neurological disorders by promoting the growth of axonals and neurites. The length and volume of nanofibers and CNTs can be manipulated, making them ideal for methods relying on biomolecule recognition and enhanced conductivity. Water-soluble carboxyfullerenes have also been used in vivo to delay motor degeneration in ALS mouse models[6]. The potential of Mesenchymal stem cells (MSCs) that were derived from human bone marrow for in vitro neuronal differentiation was investigated using poly(L-lactic acid)-co-poly-(3-caprolactone)/Collagen (PLCL/Coll) nanofibrous scaffolds. PLCL/Coll nanofibrous scaffolds allowed for 80% higher cellular proliferation rates than PLCL nanofibrous scaffolds. PLCL/Coll nanofibrous scaffold induced cellular proliferation resulted in neuronal morphologies with multipolar elongations, nestin proteins and neurofilament expression, suggesting that this method can be used for nerve regeneration[31]. Experiments involving CNT grids have suggested that the special properties of CNTs like elevated electrical conductivity can increase neuronal circuit growth transfer of neural signals where cell adhesion and dendrite elongation are supported. CNTs could potentially be used for the in vitro determination of neural network behavior which would be an essential step for designing synthetic biomaterials that could be used to repair neurological damage[32].

Other methods for restoring and enhancing neurological function that has been diminished or lost due to injury or disease involve nanotechnology based neural prosthetics. Such applications range from the acquisition of biological knowledge, the treating of neurological related illness symptoms like paralysis, deafness and blindness, and even the construction of human-machine interfaces for enhancing intellectual and athletic performance. Nanotechnology will be an essential tool in the miniaturization and integration of these implants, especially when it comes to packaging and electrode designs. Examples of neurological diseases that could be better understood and treated using neural implants include AD, epilepsy, PD, compulsory disorders as well as chronic pain and spinal cord injuries[1]. Si-multi-electrode arrays necessary for long term recording lose their electrical connectivity after being implanted within brain tissue. This loss of electrical connectivity is due to the post-implantation inflammatory reaction, which creates physical and electrical gaps between the surrounding neurons and electrode. It has been shown that bioactive nitrocellulose-based coatings can reduce electrode-brain interface inflammation in vivo by releasing anti-inflammatory agents and thereby facilitate long-term Si-multi-electrode array recordings[33]. It is also reasonable to assume that nanotechnology based neural prosthetics could be useful in treating sensory integration disorders associated with other neurological diseases like ASDs.

Femtosecond laser nanoaxotomy has allowed for a comprehensive understanding of Caenorhabditis elegans nerve regeneration with minimal effects on the worm. With the application of a nanoaxotomy chip, it was found that the distal fragments of axons that were severed through laser nanosurgery regrow while anesthetics are absent and that axonal regeneration takes place much quicker than previously thought. Nanotechnology based methods like this femtosecond laser nanoaxotomy chip will be essential tools in acquiring a deeper understanding of the biological mechanisms involved with nerve degeneration and regeneration as well as a key factor in developing human neurodegenerative disease therapies[34]. Nanotechnology has strong neuroregenerative potential.

Drug Therapies for Neurological Disorders

Nanotechnology based drug delivery methods have largely increased the effectiveness of pharmaceutical drugs in treating neurological conditions. Some drug NPs have already been sold on the market. Amorphous NPs whose dimensions either fall in a range of 20 to 30

nanometers or equal 50 nanometers have demonstrated the greatest saturation solubility increase. The optimum NP drug size depends on the necessary blood profile, administration pathway and amorphous state stability for the product's shelf life[35]. NC drugs require a special size when being delivered to and through the BBB. Their dissolution from NCs to amporphous NPs must be delayed long enough for them to reach and be internalized by the endothelial cells of their BBB target[36].

Polymer based NMs could also be used to escort neurological drugs through the BBB. Many pharmacologically active agents have not been successful in treating stroke related neurodegeneration as well as many other neurological disorders. This is due to the drugs' limited capacity in reaching and remaining stably within the target cell or tissue in vivo. Potential drug therapy targets include abnormally activated blood vessels and neurons[37]. Nanotechnology based delivery methods like implant-based delivery of drugs and NP suspension infusion with the use of catheters might be able to solve this problem[38]. Cross-linked polymer hydrogel networks known as nanogels which often combine nonionic and ionic chains have the capability to incorporate charged molecules like oligonucleotides, siRNA, DNA and low-molecular-mass compounds. These charged molecules bind to oppositely charged ionic chains which has allowed for the targeted delivery of oligonucleotides in vivo to the brain. Dendrimers have also been shown to be polymeric candidates for carrying small drugs and biomacromolecules into the CNS. Functional moieties like hydrophilic and hydrophobic polymer blocks can be attached to the dendrimer's surface to form unimolecular micelles that do not disintegrate during in vivo circulation. Dendritic boxes whose shells can only be degraded under specific physiological conditions would be suitable for targeted drug delivery and controlled drug release[6].

Polymer NPs and transport proteins like transferrin receptor antibodies have been attached to drug and signal molecules like neurotrophins to get them across the BBB. When this method was used to deliver neurophins across the BBB and into the brains of live rats suffering from artificially induced strokes, the neurotrophins reduced stroke damage to the cerebral cortex by 70%. This result suggests that drug loaded transport proteins and Polymer NPs can protect people from neurodegenerative damage and encourage neuroregeneration by promoting neuronal cell growth[39]. The transvascular delivery of short interfering RNAs (siRNAs) complexed with neuronal targeting-peptides through the BBB and into the thalamus, cortex and striatum has already been performed in vivo[40]. Candidate protein blocks can be obtained

from a library and used along with NMs like CNTs to build stable nanostructures. These nanostructures could be chemically and morphologically designed to selectively deliver drugs to specific cells[41].

Conventional Antiepileptic drugs (AEDs) are limited in their ability to treat epilepsy symptoms due to their difficulties in passing through the BBB. Drug loaded NPs have resulted in modified AEDs that can directly deliver AEDs through the BBB which have the potential to allow for novel and far more effective treatments for epileptics[42]. siRNAs that have been incorporated into gold nanorod nanoplexes have passed through in vitro BBB models[43]. It has also been shown that the modification of self-derived exosomes with targeting moieties have allowed passage across the BBB[27]. Nanotechnology will play a major role in the development of methods for the targeted delivery of drugs across the BBB.

Molecular Diagnostics for Neurological Disorders

A reasonable amount of nanotechnology related research has been performed in the area of ASD molecular diagnostics. Nanogen's nanochip based workstation has been used to identify eight MeCP2 gene mutations associated with an ASD called Rett Syndrome[44]. Atomic Force Microscopy (AFM) has been used to analyze the mechanical behavior of a protein called contactin4 which is possibly linked to ASDs[45]. DNA and proteins have been modified for use in ASD molecular diagnostic experiments involving microarrays and immunoaffinity assays. A particle based technique that combines ELISA technology with a double-antibody immunoaffinity assay has measured various biomolecule concentrations in children afflicted with either autism or Down syndrome along with a normal control group. It was found that autistic children had elevated levels of the human neurotrophin-3 protein and the vasoactive intestinal peptide. A correlation was found between the calcitonin gene related peptide and the neurotrophin-4,5 protein in autistic children[46,47]. A polyacrylamide gel-based DNA microarray has been used to successfully analyze SNPs in the brain-derived neurotrophic factor (BDNF) and gamma-aminobutyric acid receptor subunit alpha-4 (GABRA4) genes. Single SNP transmission disequilibrium tests suggested a possible link between the BDNF gene and autism. It should also be noted that involvement of the GABRA4 gene has been previously implicated in autism etiology [48, 49].

QD research that was originally used for electronic materials science is now being used in biological applications. Examples of QD experiments include the individual analysis of living

neuron glycine receptor diffusion and live animal lymph node identification during surgery using near-infrared emission[10]. Non-organic QD fluorophores have been shown to be desirable fluorescent in situ hybridization (FISH) candidates in specific mRNA transcript expression studies. A novel method that allows for modified oligonucleotide probes to undergo direct QD labeling via biotin and streptavidin interactions along with protocols for using them in multiple-label FISH. This novel technique was validated within mouse brainstem sections. QD immunohistochemical labeling was used in combination with direct QD FISH to study multiple mRNA target subcellular distribution inside the same neurons. It was concluded that FISH QDs are more sensitive than organic fluorophores and that these techniques generated superior histological results. This suggests that these methods enhance simultaneous ultrasensitive studies involving multiple protein and mRNA markers inside histological sections and tissue cultures [50]. Iron-oxide NPs that have been modified to form gold-silver alloy nanoshells could also be used to improve molecular diagnostic methods[7].

Researchers have attached a phosphorothioate-modified DNA probe to a superparamagnetic iron oxide NP (SPION). SPIONs have been used in the magnetic resonance imaging (MRI) of mouse ΔfosB and fosB mRNA in vivo after the mice were exposed to amphetamine (AMPH). Single total cDNA fragments were acquired from mouse brains acutely exposed to AMPH and amplified in vitro using reverse transcriptase-PCR for verification of ΔfosB and fosB probe specificity. Probe profiles for retention and time-dependent uptake were confirmed in GAD67-green fluorescent protein knock-in mice neurons. Signal elevation of SPION-labeled ΔfosB probes only occurred in mice that were chronically exposed to AMPH. It was concluded that the probes' target specificity in vivo allows for dependable MRI visualization for AMPH-induced ΔfosB and fosB mRNA differential elevations in live brains [51]. Nonradioactive alpha methyl tryptophan has been covalently attached to targeted magnetonanoparticles designed to cross the BBB. This nanoscopic method was used in temporal lobe epilepsy animal models to render epileptogenic tissues visible during MRI analysis [52].

Other Medical Nanotechnology Applications

Researchers have taken advantage of the intrinsic molecular recognition and self-assembly abilities of biological systems to create increasingly more advanced nanoachitectures. Nanowires that make use of templates consisting of amply engineered M13 bacteriophages have been used in the bottom-up assembly of functional nanosystems such as

heteronanoparticle architectures along with NP and NC arrays[53]. By combining techniques such as self-assembly with our understanding of the properties involving polymers and molecules at nanoscale levels, many things become possible such as plastic or molecular electronic devices, and even pulmonary or epidermal systems for delivering drugs that avoid passage through the stomach. Research is even being done on the potential development of self replicating nanomachines and scientists have already been able to manipulate cellular organelles and virus components[54]. The modification and use of retroviruses as components in the construction of nanostructures is clearly an example of an application of nanotechnology that could be used to fight neurological disorders and other diseases like cancer. Nanostructures can be used for a wide range of applications in many fields.

Many cancers affect the CNS and require treatments that can cross the BBB. Such BBB penetrating delivery systems are also essential for treating CNS disorders. Nanotechnology will allow cancer drugs to cross the BBB and increase the efficacy of cancer treatments while eliminating many undesirable side effects. NPs can be used in combination with both active and passive targeting methods, leading to increased intracellular cancer drug concentration where the normal cell toxicity is minimized. NPs can get past P-glycoprotein efflux pumps, surmounting drug resistance often found in cancer cells. Targeting moieties capable of binding tumor antigens and receptors can facilitate NP delivery to the intracellular drug action site[55]. By linking Apolipoprotein E with Loperamide and loading them into NPs made of the human form of serum albumin, Apolipoprotein E can facilitate the crossing of Loperamide through the BBB. This results in antinociceptive reactions in mice when the tail flick test is performed, indicating that the NPs have passed the BBB, showing that it can be used as an effective method of treating brain cancer and probing the brain to diagnose cancer[56,57]. shRNAs or siRNAs that were loaded onto pegylated immuno-liposomes and then systematically injected into the test subject have demonstrated impressive site specified knockdown in intracranial brain cancer models in vivo[58,59]. Aptamer-targeted NPs consisting of NCs made up of iron oxide and QDs which had been embedded into silica beads were effective in fighting cancerous brain cells and decreasing tumor sizes. Chitosan NPs that are coated with QDs can specifically target and deliver drugs to cancer cells [60].

Various studies have shown that the intratumoral delivery to the CNS using dendrimer conjugates containing anti-cancer drugs might lead to improved cancer treatment methods. These experiments demonstrated that the dendrimers' surface properties and generation are

a major factor in determining their usefulness in cancer treatments. It is also possible to create NM-cell hybrids by placing NMs inside cells. For example, immune system cells can be used as NM carriers to deliver NMs throughout the body. Mononuclear phagocytes such as microglia and macrophages have the capability to endocytose colloidal NMs and entire drug nanosuspensions. These NM-cell hybrids can then transport and release the drug at tissue sites that have been afflicted by infection, disease or injury. Multiple experiments have shown that monocytes derived from bone-marrow can carry nanoformulated drugs across the BBB and in periphery as well as to the liver, spleen, lungs and lymph nodes. Animal models have shown this treatment to be effective against HIV-I and PD[6]. Since these NM-cell hybrids travel almost anywhere in the body, they should be able to treat any type of disease, infection or injury to at least some extent. There are potential uses for nanotechnology in every area of medicine.

Ethical and Safety Issues

While nanotechnology has tremendous potential in regards to its ability to fight diseases, it also has health hazards associated with it. The large surface area of NPs makes them very bioactive and they are rapidly absorbed into cells, resulting in increased oxidative stress, which can cause the very illnesses that they are supposed to be treating such as neurological disorders and cancer. As they build up in an organism's body, NPs can also impair neuronal, respiratory and cardiovascular functions. One of the reasons for these toxic effects on biological organisms may be the result of small particles acquiring unusual surface properties after being engineered to the nanoscale. Such particles are often considered to be nuisance dusts[61]. The health risks of NPs can be reduced when they are coated with colloidal gold[62]. However, gold NPs are chemically reactive and capable of disrupting biological pathways[63], so researchers must determine if colloidal gold reduces the risks of NP enough to justify their use in clinical applications. Experiments with various NMs have identified a wide range of mechanisms that cause toxic effects on organisms.

Nanotechnology can be applied to foods and drugs in forms like food contact materials such as nanocomposites, nanostructures that enhance taste and texture and nanoscale pesticides. Some nanotechnology based foods and drugs are already FDA approved and on the market. Little is known about how the toxicology and toxicokinetics of engineered NMs (ENMs) will affect people and the environment due to difficulties in ENM characterization and detection.

The difference between ENM physicochemical properties and those of their macroscale counterparts suggests that ENM toxicity and toxicokinetic profiles cannot be determined by extrapolating data from the equivalent macroscale forms, requiring a case by case basis for risk assessment[64].

Fortunately, some progress has been made in regards to the characterization of NMs. Experiments have demonstrated that oxidative stress and reactive oxygen species (ROS) are valid test paradigms for comparing the toxicity of NPs. A wide variety of NPs were tested using these paradigms and it was concluded that cationic polystyrene (PS) nanospheres and ambient ultrafine particles induced glutathione depletion, toxic oxidative stress and ROS production. Cationic PS nanospheres also damaged the mitochondria and caused cell death with no inflammation[65]. Fullerenes are mass produced redox active lipophilic NPs that localize into regions rich in lipids like cell membranes and when in uncoated form can deplete glutathione and cause oxidative damage in fish in vivo. It has also been discovered other NPs translocate selectively from the olfactory nerves and bulb to the brain in fish and mammals[66]. Whether or not a NM is cytotoxic can depend on numerous characteristics like its type [67], shape and size. Other cytotoxicity factors include NM concentration, surfactant, molar mass distribution and surface charge along with how these factors affect cellular uptake. NPs with a positive or negative surface charge demonstrate enhanced cellular uptake and consequently greater toxicity. Also, poly(n-butylcyanoacrylate) NPs with long chains are more toxic than those with short chains and cellular uptake can be decreased when a poly(ethyleneglycol) surfactant is covalently bonded to the NP. It has been demonstrated that NPs ranging from 50 to 200 nanometers are commonly absorbed by cells at rates based on the charge and composition of cellular architecture, including BBB cells[68].

In an experiment where gold NPs that ranged from 14 to 74 nanometers in diameter were absorbed by cells by way of the receptor mediated endocytosis pathway, the maximum cellular uptake for the NPs occurred when the NPs were 50 nanometers in diameter. The quantity of gold NPs that were absorbed by cell demonstrated a decreasing trend as the NP diameter became either progressively larger or smaller than 50 nanometers. The rate at which the cells absorbed the NPs decreased over time. It was also determined that spherical NPs are taken up by cells in greater quantities than rod shaped NPs and that NP uptake is mediated by nonspecific serum protein absorption. The results of these experiments suggest that receptor recycling rates, exocytosis and receptor-ligand binding constants are mediated

by NP shape and size. It was shown by transmission electron microscopy imaging that these gold NPs became trapped in cytoplasm vesicles and did not enter the nucleus after cellular uptake. A trypan blue exclusion staining assay revealed that the gold NPs did not cause any cellular toxicity 69 (Chithrani et al., 2006), which has been reinforced by other research that claims gold NPs are less toxic than most other NPs[67]. Additional in vitro experiments with lung tumor cells have revealed that NMs in the forms of carbon nanofibers, MWCNTs, and carbon NPs exhibit size dependent toxicity. This cytotoxicity increases during functionalization of the NM surface particles after being treated with an acid[70]. These issues must be considered when using NMs in food and drug products.

Nanotechnology can be used for surveillance and other societal purposes, which raises many ethical concerns. Nanoscale surveillance devices like compact video cameras and nanotech microchips have been suggested as a method for monitoring convicted criminals and patients afflicted with neurobehavioral disorders like AD. Nanotech microchips can dispense customized drug amounts or contain assisted cognition devices capable of influencing the implanted person's behavior. The use of nanoscale devices as behavioral therapies would be very controversial, especially since such devices could eventually be used as mind control technologies. Without the proper regulations, nanoscale devices could be used to establish a panopticion society where everyone could be knowingly or unknowingly monitored and even mentally controlled through nano-panopticism [63]. Specific nanotechnology restrictions will be needed in order to maintain certain freedoms.

It is apparent that there will be many complicated issues associated with nanotechnology that will result in fierce debates and much controversy. A great deal of testing must be done to establish detailed toxicity and toxicokinetic profiles before NMs are approved for public commercial use. Ethical and safety protocols must be put in place along with careful planning in order to ensure that nanotechnology does not hurt mankind or the environment. Depending on how it is applied, nanotechnology can be a benefit or a detriment to this planet in countless ways.

Future Perspectives

Future experiments with nanotechnology will take countless different directions. It has been proposed by distinguished scientists such as Drexler, Feyman and Martel that small tools can be used to make even smaller tools, and that nanotechnology can eventually be used to

assemble molecules one atom at a time. Nanomachines can be created by specially arranging molecules of carbon and silicon. Research is also being conducted in the area of nanoscale surgery procedures for the elimination of mutant genes, and if necessary, entire genomes[62]. It has already been determined that there are ways in which many types NMs which include NPs and CNTs can safely and non-invasively be administered into the human body[6] through the vascular route. Future experiments will likely investigate the effectiveness of NMs like CNTs and NPs that have high surface–volume ratios and are capable of storing drugs for controlled drug release for use in the treatment of neurological disorders and other diseases[37].

In the near future, there will be many experiments involving gene therapies, nerve regeneration and enhanced drug delivery systems. ORMOSIL NPs that have been used to model polyQ pathologies by transferring genes into rats and mice could also be tested on primates in order to acquire a clinical symptomatology similar to human polyQ CNS pathologies. The effectiveness of ORMOSIL NPs as gene therapies can be tested in vivo on primates and ORMOSIL NP gene therapies capable of reversing human polyQ CNS pathologies could be developed[24]. While PLCL/Coll nanofibrous scaffolds have allowed for increased MSC cellular proliferation rates with neuronal morphologies, multipolar elongations, nestin proteins and neurofilament expression, more research is necessary to acquire an understanding of neuron maturation and the molecular mechanisms that result in sustained neuronal differentiation 31 (Prabhakaran et al., 2009) in order to establish a nanotechnology based system that can be used in human clinical trials for nerve regeneration. While NPs like NCs allow neurological drugs to cross the BBB, additional nanotechnology research will be needed to develop improved technologies capable of producing high dose drugs with large NC drug loads, increased solubility and a targeted or prolonged release in order to reduce side effects[35]. More work will need to be done in order to resolve safety concerns and acquire the necessary knowledge that will result in nanotechnology drastically enhancing neurotherapies.

It should also be noted that in order for these future experiments to contribute to people living longer and healthier lives, private companies will need to make much greater investments into nanotechnology. Researchers have come up with some safe[6,37] and inexpensive methods for the development of nanotechnology based diagnostics [44, 71] and drugs. There is research that could have resulted in better diagnostics and treatments for diseases like CNS disorders over 10 years ago and many scientists have complained about how their work has not resulted in nanotechnology based drugs being made commercially

available to the general public. NP based drugs and diagnostics could result in explosive profits for pharmaceutical companies so the minimal interest in nanotechnology by most private companies does not make much sense[39]. While there is a small number of nanotechnology based drugs on the market[35], the vast majority of commercially available pharmaceutical drugs do not incorporate nanotechnology based systems and continue to experience the same problems regarding side effects[3,4] and BBB penetration difficulties[2,72]. The scientific community must make a greater effort to educate private investors on the economic benefits of nanotechnology so that the necessary future experiments can be properly performed and funded. With the necessary planning and funding, nanotechnology will result in some amazing breakthroughs.

Conclusion

We have discussed in this article how a wide variety of NMs which include but are not limited to nanowires, nanofibers, CNTs, plasmid DNA and NPs like nanoshells, NCs and QDs have an enormous potential for use in neurological treatments and diagnostics. Gene therapies, regenerative techniques and drug delivery systems can be enhanced using NMs and other small scaled materials. NP-plasmid complexes, recombinant baculovirus vectors with hybrid promoters, nanofibrous scaffolds that induce neuronal differentiation in stem cells, and drug loaded NCs designed for targeted delivery across the BBB are examples of nanosystems that can be used in neurological treatments. The effectiveness of gene therapies can be tested through their ability to induce neuropathological states in animal models, which can also allow for the acquisition of knowledge about the mechanisms associated with different diseases. Other gene therapies can then be developed to treat single gene and multifactorial diseases. Nanotechnology based neuroregenerative techniques can be used to repair nerve damage and protect neurons from the effects of neurodegenerative diseases. Pharmaceutical drugs that apply nanotechnology based targeted delivery systems will yield enhanced efficacy and fewer undesirable side effects. Nanotechnology based therapies are essential for improving neurological treatments.

Applications consisting of nano-systems and advanced bioinformatics software will be used to better research, diagnose and treat countless illnesses like neurological and hyper-coagulation disorders, cardiovascular disease and cancer. CNS cancers will require treatments that can either penetrate the BBB or will be safe to use within the patient's spine.

Nanotechnology could also increase the quality of nutritional supplements and might even result in the development of self replicating nano-assemblies along with hordes of other intriguing applications. Nanotechnology research requires strict planning, precautions and regulations to ensure that the benefits of nanotechnology research outweigh its risks and costs. The toxic effects of NMs on people and the environment must be carefully investigated on an individual basis before such NMs are used in food, drugs and other industrial products. Once a nanoscale device capable of influencing behavior or being used for surveillance is implanted within someone, that person's privacy will be permanently compromised. Laws and safety protocols will need to be established to protect humanity and the environment from being hurt by nanotechnology.

There is a potentially infinite amount of ways nanotechnology can improve the quality of life for all of mankind. Nanotechnology might be used to enhance bodily components like the immune, cardiovascular and nervous systems. Eventually, researchers will use nanotechnology to regenerate the human body, allowing for the development of life extension technologies that can slow down and possibly reverse aging. By combining gene, regenerative and drug therapies with molecular diagnostics and nanotechnology, diseases like cancer and neurological disorders will either be cured or attenuated. If it is used appropriately, nanotechnology will pave the way to a brighter future.

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Correspondence Address / Yazışma adresi:
Sathees B. Chandra
Department of Biological, Chemical and Physical Sciences
Roosevelt University, Chicago, USA
847-6197968
e-mail:schandra@roosevelt.edu