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Nanotechnology based Diagnostics for Neurological Disorders

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Abstract

Kurek NS, Chandra SB. Nanotechnology based Diagnostics for Neurological Disorders. ARBS Annu Rev Biomed Sci 2012;14:1-15. Nanotechnology involves probing and manipulating matter at the molecular level. Nanotechnology based molecular diagnostics have the potential to alleviate the suffering caused by many diseases, including neurological disorders, due to the unique properties of nanomaterials. Most neurological illnesses are multifactorial conditions and many of these are also classified as neurobehavioral disorders. Alzheimer's disease, Parkinson's disease, Huntington disease, cerebral ischemia, epilepsy, schizophrenia and autism spectrum disorders like Rett syndrome are some examples of neurological disorders that could be better treated, diagnosed, prevented and possibly cured using nanotechnology. In order to improve the quality of life for disease afflicted people, a wide range of nanomaterials that include gold and silica nanoparticles, quantum dots and DNA along with countless other forms of nanotechnology have been investigated regarding their usefulness in advancing molecular diagnostics. Other small scaled materials like viruses and proteins also have potential for use as molecular diagnostic tools. Information obtained from nanotechnology based diagnostics can be stored and manipulated using bioinformatics software. More advanced nanotechnology based diagnostic procedures for the acquisition of even greater proteomic and genomic knowledge can then be developed along with better ways to fight various diseases. Nanotechnology also has numerous applications besides those related to biotechnology and medicine. In this article, we will discuss and analyze many novel nanotechnology based diagnostic techniques at our disposal today.

Keywords: nanotechnology, nanomaterial (NM), nanoparticle (NP), molecular diagnostics, bioinformatics, neurological disorder, multifactorial disorder

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1. Introduction

People are constantly being crippled mentally and physically by neurological disorders, and many of these cases are terminal. Some very deadly and debilitating neurological disorders include Alzheimer's disease (AD) (Ma *et al.*, 2004; Bishop *et al.*, 2008; Liu *et al.*, 2009), Parkinson's disease (PD) (Bilsland *et al.*, 2002; Mejías *et al.*, 2006; Peng *et al.*, 2006), and cerebral ischemia (Traystman, 2003; Dave et al., 2006; Liu *et al.*, 2007). Nanotechnology has enormous potential for helping disease afflicted people. Nanotechnology involves characterizing, designing, probing, producing and applying materials and systems where their physical dimensions are controlled at the nanoscale level (Harper, 2003; Whatmore, 2006). The U.S. National Nanotechnology Initiative defines nanomaterials (NMs) as materials with at least one dimension ranging between 1 and 100 nanometers (Oberdörster, 2004; Donaldson *et al.*, 2006). NMs can be made from a wide range of substances, including carbon, polymers, dendrimers, metals like gold and iron oxide, liposomes and even viruses (Wang *et al.*, 2009). Many materials exhibit unique mechanical, chemical, optical, electrical, magnetic and biological properties that only manifest when these materials are engineered at the nanoscale (Poma & Giorgio, 2008).

NMs can exist in many forms, which include Carbon Nanotubes (CNTs) and various types of nanoparticles (NPs) such as nanocrystals (NCs). CNTs are entirely made up of carbon, are single molecules that look like a sheet of graphite that has been rolled up into the form of a seamless cylinder, and can be classified as either multiwalled carbon nanotubes (MWCNT) or single-walled carbon nanotubes (SWCNT) (Donaldson *et al.*, 2006). It has been reported that CNTs are so strong and stiff that they can have 10 times the strength of steel and 1.2 times the stiffness of diamonds (Walters *et al.*, 1999; Yu *et al.*, 2000). When a substance is engineered as a NC, which is a crystalline NP (Elazzouzi-Hafraoui *et al.*, 2008), it demonstrates increased saturation solubility and dissolution velocity due to surface area enlargement (Junghanns & Müller, 2008). When they are of a size less than a few hundred nanometers, NPs have high surface areas per mass unit which can result in these NPs having some very interesting properties. Sometimes NPs are made up of quantum dots and other types of NCs (Whatmore, 2006).

Examples of other types of NMs include DNA (Ito & Fukusaki, 2004; Su *et al.*, 2007), nanofibers, nanoshells, nanowires, nanorods and nanospheres. While viruses and enzymes do not officially qualify as NMs, they are still very small scaled materials and are of enormous interest to many scientists that work with nanotechnology (Vázquez & Villaverde, 2010; Liu *et al.*, 2011). Viruses and proteins can be used in the synthesis of NMs (Wang *et al.*, 2009; Jain *et al.*, 2010). Viruses and proteins can also be applied in ways that are similar to how NMs can be used, which makes them appropriate materials to be included in a nanotechnology discussion (Song *et al.*, 2006; Boulaire *et al.*, 2009).

Molecular diagnostics involves the testing of DNA, RNA and proteins. It is used for a wide range of molecular biology assays and makes use of a large variety of equipment which includes gel based systems, capillary electrophoresis and fluorescent technologies. Silicon and glass microchip based immobilized oligonucleotide arrays are examples of equipment that can be used for various types of DNA analysis that include comparative genome hybridization, cDNA expression profiling, sequencing by hybridization and genetic linkage analysis. Many of these techniques involve mapping of genes along with disease-associated loci using polymorphic markers (McKenzie *et al.*, 1998; Shi, 2001; Konstantou *et al.*, 2009). RNA and DNA can be directly detected using nucleic acid tests that combine detection and amplification techniques. Qualitative nucleic acid tests for RNA and DNA include transcription-mediated amplification and qualitative polymerase chain reaction (PCR). Quantitative nucleic acid tests for RNA and DNA include quantitative PCR, real-time PCR and branched-chain DNA (bDNA) amplification (Scott & Gretch, 2007). Tests for proteins include enzyme assays that evaluate the level of function for a particular enzyme, as well as immunochemical tests that make use of antigens which can be obtained from proteins that have been purified via fractionation along with the corresponding antibodies (Kentsis *et al.*, 2009). Examples of immunochemical tests include rocket electrophoresis (Pawlaczyk & Sobieska, 2006), automated nephelometry (Joubert *et al.*, 2010) and radial immunodiffusion (Ameri & Wilkerson, 2008) as well as radiochemical and enzymatic sandwhich assays (Anderson & Anderson, 2002; Carlsson *et al.*, 2009). Common molecular diagnostic procedures include Southern blots for DNA testing, Northern blots for RNA testing, Western blots for protein testing, shotgun sequencing for genome identification using fragmented strands of DNA (Bouck *et al.*, 1998), and genome walking which can be used to sequence unknown nucleotide sequences that are adjacent to known genome regions (Leoni *et al.*, 2008). RNA sequences can be determined based on their corresponding DNA sequences.

There are many people who suffer from progressive, chronic and degenerative neurological disorders. PD affects more than 5 million people globally (Popeo & Kellner, 2009), results in motor manifestations

and other motor related symptoms, and causes depression in people that are afflicted with it (Chagas *et al.*, 2010). These motor symptoms include muscular rigidity, rest tremor, akinesia, as well as postural and balance disorders. Fatigue and insomnia are common symptoms associated with PD and also many other disorders (Goulart *et al.*, 2009). AD is the leading factor in the majority of dementia cases in patients more than 65 years old (Haes *et al.*, 2005) and is more common than any other neurological disorder (Scarpini *et al.*, 2003). AD causes the degeneration of the Central Nervous System (CNS), resulting in the loss of memory and cognitive capabilities (Parihar & Hemnani, 2004). Cerebral ischemia is a major contributor to cases of disabilities and mortalities in the United States (Dave *et al.*, 2006) along with other parts of the world (Traystman, 2003), and is positively correlated to matrix metalloproteinase-9 expression in the brain (Liu *et al.*, 2007). However, progress has been made in scientific disciplines like material sciences, molecular genetics and neurology that will eventually lead to far more effective methods for understanding and diagnosing these, and other neurological disorders. In this article, we will discuss and analyze many novel nanotechnology based diagnostic techniques at our disposal today.

2. Nanotechnology based Protein Tests for Neurological Disorders

Protein tests have been effective diagnostic tools for identifying disease afflicted individuals. For example, enzyme-linked immunosorbent assays (ELISAs) can reliably detect disease associated antibodies and antigens. A wide variety of NMs have shown excellent potential for improving protein tests. There are many examples of how nanotechnology is being used to power protein testing in order to provide better methods for diagnosing and learning about a wide variety of medical conditions, including neurological disorders.

Nanotechnology applications require the ability to make nanoscale measurements. Several methods for analyzing protein samples at the nanoscale have been developed. Nanoelectrospray quadrupole time-of-flight mass spectrometry (TOFMS) has been used to efficiently determine endogenous peptide sequences up to 9 kDa in biological samples that had previously been decomplexated via liquid chromatography. Signals are located in nanoelectrospray measurements using exact molar masses and chromatography elution behavior after using matrix-assisted laser desorption/ionization for peptidomic screening. This nanoelectrospray TOFMS method has resulted in the accurate identifications of the human 8.6 kDa cerebrospinal fluid (CSF) ubiquitin protein as well as the rat 5.0 kDa thymosin beta-4 and 4.3 kDa hypothalamic brain tissue proteins (Möhring *et al.*, 2005). Atomic force microscopy has allowed for the single molecule level study of SNARE protein mechanics involving extension, rupture force, interaction energy and spontaneous dissociation time. Consequently, it has provided greater knowledge about SNARE complex mechanisms during exocytosis (Liu & Parpura, 2009). A single-tube preparation protocol using an organic co-solvent called trifluoroethanol prevents sample loss by facilitating the denaturation and extraction of proteins. This method was far more effective in the nanoscale analysis of mouse brain tissue than traditional detergent based methods regarding the amounts of protein and peptide identifications along with the sample sizes (Wang *et al.*, 2005). Such novel nanoscale measurement methods are essential for the application of nanotechnology based protein tests.

Nanotechnology based molecular diagnostic biosensors take many forms. Nanoscale biosensors making use of NPs can be used to monitor interactions between antigens and antibodies in human CSF samples and brain extracts. These nanoscale procedures provide quantitative information on the detection of antigens and second antibodies. This information permits antigen concentration determination and aggregation mechanism analysis at applicable monomer concentrations for neurological pathogens, like the amyloid-β derived diffusible ligand (ADDL) AD pathogen (Haes *et al.*, 2005). ADDL concentrations of less than 1 pM were measured using a NP based ultrasensitive bio-barcode assay in 30 individuals' CSF. It was found that AD afflicted subjects had higher ADDL concentrations than normal subjects. Conventional protein tests cannot measure protein concentrations this low, so NP based assays are essential for developing CSF protein diagnostics for diseases like AD (Georganopoulou *et al.*, 2005). Monovalent streptavidin containing only one site for femtomolar biotin binding has been stably linked to the surface of living neurons allowing for biotinylated neuroligin-1 cross-linking-free labeling. This procedure can be applied in the introduction of a wide variety of nanotechnology based molecular probes, including quantum dots and organic fluorophores (Howarth, 2006). Many other nanotechnology based biosensor systems have been developed to enhance protein testing.

A reverse phase protein microrarray containing a laser based protein chip can simultaneously measure the activity of multiple proteins. Lasers imprint the proteins on chips that reveal whether or not the protein is active, allowing for the correlation of protein activation with the associated disease. Also,

NPs can rapidly harvest small protein biomarkers that are present in bodily fluids (Liotta & Petricoin, 2008). Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) experiments have analyzed interfacial properties between nanorod array electrodes and Fe(CN)63−/4− redox molecules. The coupling between biotin and avidin along with functionalized molecule adsorption produced capacitive and resistive changes that were indicated by EIS and CV measurements. The EIS measurement detection sensitivity was enhanced by electrodes that were modified using gold avidin-functionalized nanorods, allowing for a 1 ng/mL biotin detection limit (Lee *et al.*, 2008). The β-thymosinGln and β-thymosinHis proteins are closely related to each other and expressed in the CNS. They have been sequenced via top-down mass spectrometry (MS) in Aplysia californica, a typical neurobiology model. Nanoelectrospray quadrupole enhanced Fourier-Transform MS using electron-capture and collisionally activated dissociations have determined that both of these proteins differ only by one residue in the central actin-binding domain and are acetylated (Romanova *et al.*, 2006). Non-viral genes have been efficiently carried by organically modified silica (ORMOSIL) NPs during an attempt at modeling brain pathologies associated with polyQ peptide induced disorders like Huntington disease (HD). This ORMOSIL NP method demonstrated that polyQ type neuropathologies can still be evoked even when poly Q-extended peptide expression is restricted to nervous tissue in the adult brain (Klejbor *et al.*, 2007). Nanotechnology based protein tests can be very effective when it comes to learning about how proteins affect disease progression and in diagnosing neurological diseases.

Fluorescent dye-doped NPs have been one of the most effective NMs in protein microarrays and are also more useful in other bioassay systems involving immunohistochemistry, immunocytochemistry and fluorescent-linked immunosorbent assay. Ultrasensitive target detection was accomplished when the specificity of antibody-mediated recognition was combined with high-intensity luminescent NPs (Lian *et al.*, 2004). Near-infrared range (NIR) fluorescent oligodeoxyribonucleotide (ODN) reporters with the ability to sense NF-κB p50 protein transcription factor binding have decent potential for analyzing protein-DNA interactions (Zhang *et al.*, 2008). Fluorescent genes have been loaded onto NPs and carried on bacteria surfaces. The bacteria were incubated within cells and fluorescent signals were detected when the genes from the NPs were expressed in the cells, allowing for the monitoring of the protein production that was associated with these genes (Akin *et al.*, 2007). Fluorescent NMs will potentially be essential diagnostic tools.

3. Nanotechnology based Genetic Tests for Neurological Disorders

Genetic diagnostics like DNA and RNA tests can be used to acquire information about the molecular mechanisms associated with an individual patient's disease (Braziel *et al.*, 2003). Genetic diagnostics are also important for treating, diagnosing and predicting the onset of diseases as well as identifying carriers of the diseases. In order to develop a clinical test or treatment for a genetic disease, large families are first studied in order to identify familial diseases along with the genomic loci that become segregated with the diseases. Once a gene associated with a disease is identified, further studies determine how mutations in the gene impact the clinical variability of the disease process's pathophysiology, which can lead to better ways to treat, diagnose (Van Deerlin *et al.*, 2003) and monitor therapy for the disease (Amos & Patnaik, 2002). Quantum dots, silica and metal NPs have been tested for their potential as genetic probes (Zhao *et al.*, 2003). Additional NMs like DNA, electrical and optical biosensors along with other small scaled materials like enzymes (Song *et al.*, 2006) and viruses (Boulaire *et al.*, 2009) have also been investigated for use as genetic probes. These materials can be applied to nanotechnology based genetic tests for diseases like neurological disorders in many ways.

Superparamagnetic iron oxide NPs (SPIONs) linked with randomly sequenced oligodeoxynucleotides (ODNs) and phosphorothioate-modified ODNs (sODNs) complementary to either β-actin mRNA (SPION-β-actin) or c-fos mRNA (SPION-cfos). These SPIONs have been used as probes in magnetic resonance imaging (MRI) for tracing cerebral ischemia gene transcripts in living animals. The SPION probes were introduced into male mouse left cerebral ventricles by intracerebroventricular infusion. A significant increase in SPION-cfos retention was found in areas where ischemic insult triggered an increase in c-fos mRNA. This suggests that SPION based MRI methods can be used in vivo to target gene transcripts associated with acute neurological diseases (Liu *et al.*, 2007). When used in conjunction with Affymetrix high density array chips, recombinant baculoviral vectors capable of efficiently transducing brain cells have been used to profile gene expression. These recombinant baculoviral vectors have identified subsets of genes involving cytokine-cytokine receptor interaction and Toll-like receptor signaling that could

potentially be indicators of baculoviral transduction induced molecular responses in neural cells (Boulaire *et al.*, 2009). Inexpensive polypropylene micro reactors have allowed for nanoliter scale liquid PCR-based assays that have been able to analyze RNA expression of four mouse brain genes (Dahl *et al.*, 2007). There are many more examples of how nanotechnology related techniques can be used to monitor, diagnose and acquire genetic knowledge about diseases like neurological disorders.

In a novel assay, target DNA concentrations as low as 50 femtomolars were detected from amplified signals using a process involving silver reduction reactions catalyzed by gold NPs when the target was hybridized by a DNA strand (Taton *et al.*, 2000). Gold NPs have been able to quantify DNA while discriminating between single nucleotide polymorphisms due to definitive NP-DNA conjugate group formations present with target DNA. The conjugate groups were quantitatively characterized using gel electrophoresis (Qin & Yung, 2007). The effects of spacer composition, salt concentration, NP size and sonication degree were tested on gold NPs regarding their effects on the NPs' DNA coverage. Maximum loading was acquired by NPs in the presence of DNA possessing a poly(ethylene glycol) spacer that were salt aged to approximately 0.7 M NaCl. Sonication of the NPs during surface loading substantially increased DNA loading. NPs with larger surface areas displayed greater DNA loading than those with smaller surface areas (Hurst *et al.*, 2006). Phase behavior manipulations involving colloidal gold interaction potentials can also result in more accurate and sensitive DNA detection methods. It has also been found when using optical absorption spectroscopy that the phase transitions and optical properties of DNA-gold NP assemblies can be manipulated by adjusting the DNA sequence, NP size, length, density, electrolyte concentration and interparticle distance (Sun *et al.*, 2007). The unique properties of gold NPs make them an interesting candidate for genetic analysis.

Experiments have demonstrated that fluorescent dye-doped NPs are more effective than quantum dots and other fluorescent labeling agents in detecting DNA and other biological molecules (Lian *et al.*, 2004). Dye-doped silica bioconjugated NPs capable of producing strong fluorescent signals when properly excited have also allowed for more sensitive, accurate and selective genetic analysis methods. This is due to their ability to encapsulate large numbers of fluorophores, high photostability that is caused by the silica matrix shielding effect, and silica surface that acts as a versatile and universal biocompatible substrate for biomolecule immobilization (Santra *et al.*, 2001; Santra *et al.*, 2001; Zhao *et al.*, 2003). Fluorescent dye-doped NPs have allowed for the visualization of target RNA binding probe hybridization signals on Affymetrix GeneChip® arrays. These NPs are less expensive and more reproducible than traditional probes, produce a stronger fluorescent signal than the streptavidin-phycoerythrin (SAPE) protein, and have potential uses for quantitative genetic diagnostics (Wang *et al.*, 2007).

Fluorescent probes have also taken other forms. NIR fluorescent ODN reporters capable of detecting NF-κB p50 protein binding have displayed efficient fluorescence resonance energy transfer. The donor and acceptor fluorochromes used in this method interacted with each other without interfering with base pairing due to the use of hydrophilic internucleoside phosphate linkers, allowing for DNA-protein interaction detection (Zhang *et al.*, 2008). Micrometer-sized two-dimensional arrays that are capable of achieving multiplexed detection due to their ability to carry barcoded fluorescent dyes and nucleic acid probes have been created through the combinatorial self assembling of DNA nanotiles. These arrays can rapidly detect multiple aptamer binding molecules and DNA sequences by accurately controlling solution-based binding reactions and interprobe distances (Lin *et al.*, 2007). A significant amount of research has shown that fluorescent NMs are good candidates for use as genetic probes.

4. Nanotechnology based Molecular Diagnostics Applications for Bioinformatics

Bioinformatics is a new and important biotechnology field. Bioinformatics involves the use of computer programs and on-line data banks to manipulate and store information involving genetic and protein sequences while converting related data into useful information. The acquisition of proteomics and genomics knowledge that is sufficient for scientific progress requires the use of complex bioinformatics computer software. Data obtained from various fields has been harmonized to better understand protein and gene expression information. The Integrative Gene and Protein expression (InGaP) database is part of a continuing cDNA project and contains comprehensive profiles associated with 127 mKIAA proteins and genes related to theoretical ones. KIAA cDNAs and their mKIAA cDNA mouse counterparts have been isolated from brain tissue derived cDNA libraries. The associated genomic and proteomic data can be integrated and manipulated for research by the neuroscience community (Koga *et al.*, 2004). The InGaP

database is one of many examples of how large amounts of genetic and protein related data can be maintained, organized, manipulated and made available for public use. A tremendous amount of proteomic and genomic data can be obtained using nanotechnology based molecular diagnostics and then processed for further applications using bioinformatics. After being interpreted and manipulated using bioinformatics software, this data can then be applied to additional nanotechnology based research. These additional applications can lead to even greater knowledge and the development of revolutionary treatments for diseases like neurological disorders.

Novel paradigms are being devised in systematic bioinformatics synthesis in order to fabricate nanotechnology applying systems. This will result in the derivation of new operation principles for examining subsystem functionality as well as researching complex architectures and novel structures in regards to the nanoarchitectronics horizon. Nanosystems designed with bioinformatics engineering paradigms have decisively identified the patterns and periodicity in the designs of distinct biosystem components. Entropy and correlation analysis that were performed on these nanosystems indicated that the template patterns need to be investigated while providing viable methods for procedures involving functionality analysis, pattern recognition, synthesis, optimization, prototyping and redundancy (Lyshevski *et al.*, 2003). Such nanosystems are necessary for the acquisition of bioinformatics data when analyzing extremely small biomolecules. When looking for neurological peptide disease biomarkers with molecular masses of less than 10 kDa, a decent amount of specific fragments are necessary for successful database searches. Nanoelectrospray quadrupole TOFMS methods provide high-quality data that is ideal for the determination of peptide sequences in brain and spinal tissue. Protein precursors, post-translational modifications and proteolytic processing can be identified using automated database searches (Möhring *et al.*, 2005). With the proper nanotechnology based procedures and paradigms, innovative bioinformatics software can be developed and allow for exponential progress in the area of biotechnology.

There are many examples of how nanotechnology applying systems along with their associated bioinformatics software have resulted in a revolution in biotechnology research. Nanogen's Nanochip system integrates a reader consisting of computerized data analysis hardware and software along with a loading device that adds patient samples to a cartridge. Nanogen's system allows for efficient genetic analysis where the results can be displayed within tables and histograms. The Nanochip system accelerates the molecular binding process by a factor of 1000 when compared to traditional systems and electronically addresses biomolecules to specific test sites where fluorescence is measured using two lasers once stringency is achieved. This accelerated molecular binding process can be used to rapidly acquire bioinformatics data involving insertions, deletions, single-nucleotide polymorphisms (SNPs) and short tandem repeats in genes like apolipoprotein E, which is associated with AD (Jannetto *et al.*, 2004) and possibly multiple sclerosis (Zhang *et al.*, 2010). Roche's LightCycler uses thin borosilicate glass capillaries to rapidly complete genomic DNA PCR amplification cycles. During this accelerated PCR process, two probes are hybridized to neighboring amplicon areas, which produce a fluorescent signal that decreases as temperature increases due to the hybridized probes' dissociation. This system utilizes computer software to generate graphs of fluorescence intensity vs temperature along with the derived melting curves. Melting curves with highly reproducible sequence specific dissociation temperatures where earlier dissociations result from non-perfect matches caused by genetic mutations can readily distinguish wild type samples from homozygous or heterozygous sequences (Schrijver *et al.*, 2003). Much bioinformatics data can be acquired regarding mutations identified by these systems.

There are other nanosystems that can also be effective in obtaining bioinformatics data. Enzymatically generated peptide profiles from microdissected brain blood vessel samples have been analyzed using a MALDI-FTMS technique involving nano-LC fractionated samples. This technique has resulted in the acquisition of proteomics data regarding the identification of differentially expressed proteins. The data obtained from the MALDI-FTMS technique was analyzed using the DataAnalysis software package and the Sophisticated Numerical Annotation Procedure algorithm (Mustafa *et al.*, 2007). The Oral Fluid NanoSensor Test (OFNASET) utilizes self-assembled monolayers, cyclic enzymatic amplification, bionanotechnology, molecular purification and many other novel technologies including computer software. The OFNASET generates charts like column graphs that display RNA and protein concentrations in pg/ml along with relevant statistical information. Nanosystems like the OFNASET are essential tools for acquiring bioinformatics data while definitively identifying neurological and cancer associated genomic and proteomic biomarkers (Gau & Wong, 2007). Single-molecule fluorescence microscopy is a nanoscale technique that utilizes the Förster resonance energy transfer phenomenon. This method has been extremely useful in acquiring bioinformatics data involving protein structure dynamics

associated with protein misfolding like homogeneousity and time scale measurements. Protein misfolding is linked to neurodegenerative disorders like AD, Down's syndrome, HD and PD (Uversky *et al.*, 2006). A bioconjugated sandwich assay based on dye-doped organic silica NPs capable of discriminating between DNA strands differing by only one base pair can be applied for ultrasensitive DNA analysis. This assay can be used to group together DNA sequences in bioinformatics databases that differ by as little as one base pair (Zhao *et al.*, 2003). Nanotechnology based diagnostic systems can acquire unique bioinformatics data through many different methods.

5. Multifactorial Neurobehavioral Disorders

Many neurological conditions follow a complex inheritance pattern and are referred as multifactorial disorders because they involve interactions between multiple genes and environmental factors. The symptoms of multifactorial conditions are usually irreversible and emerge once certain thresholds are reached. Some disorders also cause irregular behavioral patterns due to damage or abnormalities in the patient's nervous system, so researchers refer to these disorders as neurobehavioral disorders. Most of the disorders previously discussed in this article are multifactorial neurobehavioral disorders and other examples include several forms of epilepsy (Khan & Jinnah, 2002; Price *et al.*, 2009), schizophrenia (Vyas *et al.*, 2010) and autism spectrum disorders (ASDs) (Rinehart *et al.*, 2002; Green & Flanagan, 2008). These disorders deserve special attention in the area of nanotechnology.

Some of the many different forms of multifactorial epilepsy are also classified as neurobehavioral epilepsies. Epileptics often suffer from mood disorders, sexual dysfunction, episodic psychosis and cognitive problems like memory loss (Blum & Bortz, 1998). Epilepsies are seizure disorders that affect approximately 50 million people and cause considerable pain, suffering and disability (Reyes & Parpura, 2008). Ion channels regulate electrical activity in the nervous system. Epilepsies result from abnormal electrical activity that affects either the entire brain or only a focal area. Fluorescent nanosensors can provide real-time measurements and images of sodium because they are completely selective and are reversible over other cations like potassium. Nanoscopic techniques involving fluorescent nanosensors have determined sodium channel activation induced by drugs in heterologous cells after transfection with voltage-gated sodium channels. These nanosensors provided cellular insight into how small functional variations in sodium channels can have dramatic effects on action potentials (Dubach *et al.*, 2009). Nanoscopic techniques have allowed membrane ion channels in the peripheral and central nervous systems to be characterized in regards to their dynamics, morphology and physical properties. Atomic Force Microscopy (AFM) has been combined with the patch clamp technique in order to characterize single mechanosensitive ion channels and to better understand channelopathy pathological mechanisms (Lehmann -Horn & Jurkat-Rott, 2003). Nanoscopic techniques like these can be applied to learn more about seizures and develop better epilepsy treatments.

Schizophrenia is a multifactorial neuropsychiatric condition that has sparked a great deal of interest. Multiple studies have reported a significant loss of grey matter during the onset of schizophrenia (Pantelis *et al.*, 2010). Mutations that effect FOXP2 gene expression are commonly found in schizophrenics, which suggests an association between schizophrenia and the parts of the brain involving language development (Tolosa *et al.*, 2010). In 4 percent of cases, the symptoms of schizophrenia become apparent before the age of 18 and such early onset cases display developmental delays in speech, motor skills, language and social interactions. In 96 percent of cases, the symptoms of schizophrenia become apparent later on in young adults that are at least 18 years old. Common symptoms of schizophrenia that are displayed in both early and later onset cases include cognitive difficulties such as impaired memory, learning and intellectual functioning (Vyas *et al.*, 2010) as well as audio and visual hallucinations, delusional thinking, paranoia and other psychotic behavioral patterns.

Information that is critical for diagnosing and monitoring the progression of brain diseases like schizophrenia can be obtained from putative cerebrospinal fluid (CSF) biomarkers using nanotechnology based LC-MS/MS methods. The initial study involving this method for detecting CSF biomarkers allowed for the accurate detection of 77 CSF proteins, and 7 of these proteins had not been previously identified. Schizophrenia patients differed profoundly from normal people in their CSF protein compositions (Huang *et al.*, 2007). A peptide correlation analysis that made use of similar nanotechnology based LC-MS/MS methods accurately reflected relative abundances of proteins in largely scaled sample sets of both spiked and clinical serum from both normal and schizophrenia afflicted people (Schwarz *et al.*, 2007). Another study involving these quantitative label-free proteomic platforms allowed for the identification of 1709

serum proteins over a dynamic range consisting of more than 3 orders of magnitude (Levin *et al.*, 2007). Nanotechnology based diagnostics such as these can be extremely useful in the development of pharmacogenetic medications and other novel treatment methods.

ASD incidence rates have rapidly increased, sparking intense research involving both the causes of ASDs and the development of better ways to treat ASD afflicted patients. The genetic component of ASDs accounts for 80% of the liability variation with genetic etiologies that are common to other neuropsychiatric conditions (Lichtenstein *et al.*, 2010). Rett syndrome (RS) is a very debilitating ASD (Guy *et al.*, 2007). RS involves the gene known as methyl CpG binding protein 2 (MeCP2) and is almost exclusive to females (Hardwick *et al.*, 2007; Venâncio *et al.*, 2007). Some symptoms of RS involve the loss of communication skills, breathing difficulties, and the occurrence of gait apraxia (Thistlethwaite *et al.*, 2003). Asperger's syndrome (AS) is an ASD where the afflicted person has average or high intelligence along with rigid thinking and normal language development. The 3p21–24 locus is a primary candidate for fine scale mapping in AS research. Some of the symptoms commonly found in people with ASDs like AS and RS include poor eye-hand coordination and motor skill deficiencies, insomnia, OCD tendencies, prosopagnosia, sensory abnormalities and impaired social interaction (Rehnstrom *et al.*, 2006). Other ASD symptoms include intense sensitivity to physical pain, hearing impairment, hand flapping, sustained odd play, toe walking (Mandell *et al.*, 2005), tics (Mejia & Jankovic, 2005) and convulsions (Parmeggiani *et al.*, 2010). Extensive research has been done on ASDs and there is even more research that still needs to be done in these areas.

Some of this ASD research involves nanotechnology. The Nanogen workstation consists of a thermal discrimination and electric hybridization based two-color assay performed on a nanochip that is electronically active. Nanogen's workstation allowed for the accurate, specific, fast and cheap identification of seven of the eight MeCP2 gene mutations that have commonly been associated with RS. This method involved the linking together of two amplimers along with the serial hybridization of seven loci (Thistlethwaite *et al.*, 2003). The mechanical behavior of a protein that is suspected to be associated with ASDs called contactin4 (CNTN4) has been determined using a technique that combines AFM and a simulation involving steered molecular dynamics. This AFM method was applied during CNTN4's folding process and several observations were made. It shown that CNTN4 exibited modular force curves typically found in modular proteins with no more than four unfolding peaks and the occurrence of three unfolding lengths were confirmed. Small plateaus that probably resulted from forced transitions inside domains were also observed (Strzelecki *et al.*, 2009). Nanotechnology based diagnostics will be helpful in identifying both the genetic and environmental factors that contribute to the onset of ASDs, epilepsy and schizophrenia and in developing treatments for people that have been afflicted with these conditions.

6. Other Medical Nanotechnology Applications

Other applications concerning nanotechnology based molecular diagnostics and pharmocogenomics include SNP tests for hypercoagulation disorders along with assays involving infectious agents and cancer. Nanosphere's Verigene platform, which uses stable, non-toxic gold NPs and silver for signal amplification, is one of many systems capable of performing such assays (Jannetto *et al.*, 2004). A biomimetic nanochannel system with an ion concentration effect which generates a nonlinear potassium ion response at concentrations in the range of 0 to 1500 μM can be used to efficiently study conformational changes in biomolecules by measuring the current in confined areas. This nanochannel system has the potential to be used for the study of ion channels in vivo (Hou *et al.*, 2009). Nanotechnology tools that can be used to study ion channels and disease-associated genomic and proteomic biomarkers are also necessary for enhancing both neurological and cancer diagnostics.

Nanotechnology based diagnostics have demonstrated enormous potential in the fight against cancer. After microdissection, nano-LC fractionated glioma and normal brain blood vessels have been analyzed using MALDI-FTMS in order to measure their enzymatically generated peptide profiles. MALDI-FTMS analysis of these blood vessels has resulted in the identification of four proteins exclusively expressed in glioma blood vessels. MALDI-FTMS analysis proved that gel-free MS methods can be used to successfully distinguish between differentially expressed proteins in small cell samples obtained from microdissection procedures (Mustafa *et al.*, 2007). The OFNASET integrates breakthrough technologies to definitively identify disease-associated genetic and protein biomarkers. It offers simple and cheap sampling techniques that minimize the subject's discomfort and utilizes a quantitative and accurate diagnostic system. The OFNASET is a point of care platform intended for the multiplex detection of oral cancer

salivary biomarkers that has already provided highly specific and sensitive oral cancer detection with the use of four RNA and two proteomic biomarkers (Gau & Wong, 2007). Reverse phase protein microrarrays can determine the proteins that are active inside a cancerous cell using laser based protein chips. NPs can harvest cancerous biomarkers from bodily fluids. Nanotechnology based diagnostics can determine the most effective drug for fighting the individual's cancer based on the active proteins from the tumor, paving the way for personalized chemotherapy treatments (Liotta & Petricoin, 2008).

Nanotechnology can be used in countless ways in just about every industry. Many of these nanotechnology applications will eventually be used for medical purposes, like treatments and diagnostics for diseases such as cancer and neurological disorders. Protein and genetic tests for cancer can be modified to diagnose neurological diseases. Nanotechnology has the potential to improve our lives in an almost infinite capacity.

7. Nanomaterial Characterization and Synthesis

A reasonable amount of research involving the characterization and synthesis of NMs has already been completed. Two methods for polymeric NM synthesis are photolithography, and laser ablation technology. It was concluded that interactions in a liquid polymer resin media between lasers and polymeric NPs cause light scattering due to changes in the media's refractive index (Kavadia, 2009). Certain NPs, like titanium dioxide NPs, are good at absorbing UV light (Whatmore, 2006). It has also been shown that when free electrons interact with light in gold or silver nanostructures, collective oscillations referred to as surface plasmons (SPs) can occur. In general, the extinction spectrum for smaller NPs with a radius less than 20 nanometers is dominated by light absorption while light scattering is the dominant process for larger NPs (Xia & Halas, 2005).

Silver nanostructures have been synthesized in large yields consisting of an assortment of various well-defined shapes using polyol reduction. It was concluded that gold nanocage surface plasmonic resonance peaks can be shifted between 425 and 1200 nanometers by adjusting the molar ratio for Ag and HAuCl4 (Wiley *et al.*, 2005). NP cross sections for absorption and scattering can be obtained once the specification of each element's polarizability and location has been established. As the NP size becomes larger, the SP peak tends to shift toward red wavelengths and new peaks might also show up in the extinction spectra because of quadrupole mode excitation. The scattering and absorption cross sections for silver and gold NPs have been shown to be 5 to 6 orders of magnitude higher than those of molecular species like organic chromophores. The wavelengths along with the scattering and absorption coefficient magnitudes for silver and gold NPs can be controlled by specifically engineering their shapes, dimensions, structures and geometric parameters. In an experimental example, gold colloids with a radius of 20 nanometers showed greater absorption and less scattering of light than those of a 50 nanometer radius. The scattering and absorption peaks for gold colloids with a radius of 50 nanometers also varied over a much wider spectrum of wavelengths than those with a radius of 20 nanometers (Xia & Halas, 2005). A NM's ability to scatter or absorb light can vary greatly depending on the type of NM along with its synthesis method, physical characteristics and properties.

CNT conductivity is another topic that deserves discussion. CNTs have either semiconducting or metallic properties depending on the CNT's chirality (Che *et al.*, 2000), with metallic forms having 1000 times the electric conductivity of copper (Whatmore, 2006). It has been shown that channel resistance dominates diffusive carrier transport in semiconducting CNT transistors that have channel lengths greater than 300 microns. Semiconducting CNT transistor field-effect and intrinsic mobility value estimates exceed all known semiconductor values (Durkop *et al.*, 2004). 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 (Snow *et al.*, 2003). NMs have interesting properties.

NMs must be characterized in regards to their cytotoxicity when ingested and absorbed (European Food Safety Authority, 2009). Experiments have shown that NM cytotoxicity can depend on the NM's size (Magrez *et al.*, 2006), shape (Chithrani *et al.*, 2006), concentration, molar mass distribution, surfactant, surface charge (Mailänder & Landfester, 2009) and type (Male *et al.*, 2008). Strict safety protocols must be followed in applications involving NMs.

8. Past and Future Perspectives

Nanotechnology research has come a long way over the past 50 years and there is much more work that still needs to be done. The field of nanotechnology originated in 1959 when a physicist named Richard Feynman realized that it is possible to manipulate and probe individual atoms and molecules at nanometer scales (Gilmore *et al.*, 2008). Other important advancements in nanotechnology include the invention of the scanning probe microscope in 1981, discoveries of the carbon-60 molecule in 1985 (Whatmore, 2006), the fairly recent capability of scientists to control the NP sizes (Harper, 2003) and the discovery of the potential of CNTs by Sumio Iijima in 1991 (Whitby *et al.*, 2004). It has also been recently discovered that NP toxicity can be greatly reduced by coating NPs with gold (Hede & Huilgol, 2006). These discoveries along with other recent advancements in the area of nanotechnology will allow for future research to be done in regards to the development of diagnostics, medicine, electronics and many other products in nearly every industry.

Future experiments involving nanotechnology based protein tests will utilize systems like the reverse phase protein microrarray. This system could be enhanced so that it could be used in vivo to test for early indications of disease by capturing super low biomarker concentrations while the NPs circulate in the patient's bloodsteam. After enough time passes, a blood sample can be taken from the patient and the NPs can be analyzed for early signs of disease, making it a valuable tool for the diagnosis and prevention of other diseases besides cancer like AD (Liotta & Petricoin, 2008). Fluorescent NIR ODN reporters have the potential to directly detect protein-DNA interactions. They can be further optimized in regards to the respective positions of the acceptor and donor fluorochromes on the base pairs constituting the binding sites. Researchers could then record cognate interactions between hairpin-like probes or specific double-stranded DNA duplex and transcription factors in living cells and systems (Zhang *et al.*, 2008). A NP based ultrasensitive bio-barcode assay that can measure extremely low ADDL concentrations in CSF for the detection of diseases like AD could also be modified to detect and study a large variety of other CSF pathogenic biomarkers. This will lead to experiments designed to diagnose a wide range of neurological diseases. This bio-barcode assay should also be statistically evaluated with a larger population of patients (Georganopoulou *et al.*, 2005). These protein diagnostic nanosystems can be expanded to diagnose many different neurological diseases.

Future experiments involving nanotechnology based genetic tests will rely heavily on systems that do not require PCR amplification like Nanosphere's Verigene platform. Technologies that eliminate the need for genetic amplification procedures like PCR will not be subject to expenses and will lessen the labor and turnaround time required to perform genetic diagnostics. This will allow for the development of personalized pharmacogenomic medicine based on an individual's genotype (Jannetto *et al.*, 2004). Nanogen's NanoChip Molecular Biology Workstation provides a cheap method for detecting SNPs and point mutations associated with neurological disorders like Rett Syndrome. Additional experiments that use the same chip to multiplex amplimers can be designed to further reduce this system's cost (Thistlethwaite *et al.*, 2003). Fluorescent dye doped NPs allow for simplified staining procedures along with improved photostability and detection sensitivity when used as Affymetrix Genechip staining probes. Optimization of the NP size, Axon scanner optics and single-wavelength laser diodes or the synthesis of dye-doped NPs that match the PE Ex/Em wavelength will be necessary for precisely diagnosing single gene and multifactorial diseases (Wang *et al.*, 2007). With more research, genetic diagnostics can become cheap and accurate enough to diagnose countless neurological disorders.

There is still much work that needs to be done before nanotechnology based molecular diagnostic systems become commercially available to the public. However, with the proper research and funding, nanotechnology based diagnostics will be used to objectively and accurately diagnose neurological disorders and other diseases that are currently being diagnosed on a subjective basis. Nanotechnology based diagnostics along with specialized bioinformatics software can be used to acquire a much deeper understanding of the mechanisms associated with diseases like neurological disorders. This will result in far more effective treatments for such diseases than what is currently available. Future experiments involving nanotechnology will seek to drastically improve the medical industry.

9. Conclusion

Nanotechnology is truly an amazing field. Over the past 50 years, many different types of NMs have been discovered and much knowledge about nanoscience has been acquired. The unique properties

exhibited by NMs will exponentially enhance diagnostics and treatments for neurological disorders and other diseases like cancer, hypercoagulation disorders and conditions caused by infectious agents. Nanotechnology based molecular diagnostics along with specialized bioinformatics software will allow researchers to acquire hordes of disease related information and other biological knowledge. This will lead to the establishment of personalized treatment plans for preventing and treating diseases.

NMs like nanochips, nanotiles and plasmid DNA along with iron oxide, gold, silica and fluorescent dye doped NPs have resulted in the development of advanced nanotechnology based molecular diagnostic systems that utilize specialized bioinformatics software. Nanoplatforms such as the reverse phase protein microrarray, Nanogen's NanoChip Molecular Biology Workstation, and Nanosphere's Verigene Platform along with other nanosystems like recombinant baculoviral vectors that use Affymetrix high density array chips for cellular transduction are examples of such nanotechnology based diagnostic systems. These and other previously discussed nanosystems have allowed for significantly more accurate, timely, specified, sensitive, quantitative and cost effective methods for analyzing and detecting specific proteins and genetic material present in low concentrations within biological samples.

The NMs that we have discussed in this article will provide a nearly endless amount of research in almost every industry. NMs may someday be used to cure cancer or as components in the construction of more complex nanostructures. Nanotechnology will be a critical component in the development of improved diagnostic procedures and life extension technologies. Over time, we can expect to become increasingly dependent on nanotechnology to meet our needs, whether these needs involve advanced computer chips, food and nutritional supplements, cosmetic products, automobile fuels, or even household appliances, decorative and cleaning products. Nanotechnology will improve everyone's quality of life in a countless number of ways.

There clearly exists much potential for using nanotechnology to understand, prevent and diagnose neurological disorders like AD, PD, cerebral ischemia, epilepsy, schizophrenia and ASDs such as Rett Syndrome along with many other diseases like cancer. As long as nanotechnology is applied with the appropriate precautions, the benefits that make nanotechnology preferable to traditional methods of diagnosing diseases far outweigh its risks. Research involving the use of nanotechnology to fight illnesses such as the neurological disorders that we have discussed in this article is worth its cost. Nanotechnology based diagnostics will lead to advanced disease treatments that will make the unbearable pain and suffering these afflictions cause a distant memory.

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