

**APPLICATIONS OF NANOTECHNOLOGY IN THE
DIAGNOSIS AND TREATMENT OF PARKINSON'S
DISEASE**

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Abstract

Nanotechnology is one of the most new and innovative developments in contemporary science and is set to revolutionise the future of medicine. Vast discoveries and huge advances have been made in this field over the past decade and nanomedicine has earned its right as a valid and profound subdivision of nanoscience. This paper will discuss uses and applications of nanotechnology in modern day medicine, particularly concerning Parkinson's disease, and propose further developments to the current techniques. It will also consider various nanotechnological methods which could possibly be altered, modified or implemented in the future. To conclude, the ethics of using nanotechnology in medicine will be reviewed.

Introduction

The word "nano" is derived from the Greek "nanos", directly translating to English as "dwarf". From the very literal meaning of the word, therefore, it is inferred that the science is operated on a miniature scale. When working at a nanoscale, the prefix "nano" is used as an SI unit to denote any value multiplied by 10^{-9} , meaning that a nanosecond is roughly a billionth of a second and a nanometre equates to a billionth of a metre. Ten hydrogen atoms lined up side by side would equate to a length of 1nm; in fact, most atoms are a miniscule 0.1-0.2nm wide. Due to the fact that work on a cellular level is done primarily at a nanoscale, it is therefore unsurprising that nanotechnology has paved the way for a vast quantity of biological development, and a great majority of the findings encountered have led to cutting edge breakthroughs in this area.

Nanotechnology offers various exciting prospects to every aspect of life. From dietary supplements to clothing, nanotechnology is evolving rapidly all around us. It offers numerous possibilities, especially in the medical sector of nanoscience (nanomedicine).

Nanomedicine can be defined as "the design and manipulation of nanoparticles, particularly as applied to the medical diagnosis and treatment of disease." Examples of recent nanomedical developments include; the use of nanoparticles with antibacterial properties in hospital equipment and the development of magnetic nanoparticles being used to target disease, reducing the necessity of surgery and the associated risks. A further innovation in nanomedicine has been manufacturing drugs as nanoparticles as they are thought to be absorbed more easily into the body because of their size. It could offer easier methods of locating and targeting specific cells on a 'nano' size level, on an atomic scale, and delivering drugs to these cells. This is good because often very powerful drugs are needed to kill mutated cells such as tumour cells, and these drugs would be hazardous if they came into contact with normal functioning cells.

Over 200 years ago, James Parkinson first described a group of symptoms that later became known collectively as "Parkinson's disease". Modern day science has allowed us to improve our understanding of the disease significantly and develop treatment that makes a considerable difference to how people cope. However Parkinson's disease remains a massive struggle for those who suffer from it and also to the doctors and other medical professionals who are involved in its management. The main obstacle of the disease is that there is no way of curing it currently; treatment can only offer temporary symptomatic relief. Also, as of yet, there is no way of knowing why people develop the disease and it is still being debated as to whether or not certain people have a greater affinity for developing Parkinson's than others.

Parkinson's disease is a progressive neurological condition seen in the United Kingdom (UK) at a prevalence of 1 in every 500 people. Parkinson's is a common illness that has a huge impact on the UK's population and health service. People over the age of 50 are the most likely to develop the disease but 1 in 20 who develop the disease is under the age of 40. It is vital to develop effective treatment strategies. Nanotechnology is a new progression in medicine which may be used to help treat this common disease.

Parkinson's occurs in people who do not have enough of the chemical dopamine, because some of the nerve cells which produce this neurotransmitter in their brain are no longer functioning. This causes patients to move more slowly and thus it takes them longer to perform everyday activities. The symptoms of Parkinson's tend to get worse as time passes, but the disease does not directly cause people to die. Symptoms of the disease include; slowness of movement, rigidity and tremors. However, people with Parkinson's can find that tiredness, pain, depression and constipation also affect their day-to-day lives. The symptoms that affect someone with Parkinson's disease and how quickly the condition develops vary from patient to patient.

Diagnosis of Parkinson's disease is not straightforward because there is no specific test to confirm the condition and therefore it needs to be diagnosed by a specialist. The symptoms can be controlled by prescribing pharmaceutical drugs, a variety of therapies and also surgery in very rare cases.

Discussion

Recent Nanotechnological Developments in Diagnosis of Disease

In recent years, as the medical uses of nanotechnology have escalated in terms of both variety and frequency of use, much new advancement has been made, particularly in relation to diagnosis. One of the most recent and influential innovations in this sphere is the discovery and creation of nano-sized chips, with the potential to diagnose disease rapidly, commonly referred to as "Lab on a Chip" (see figure 1). Since the 1990s, a great amount of research has been carried out into the new-found lab-on-a-chip discovery, and as specialised scientific instruments have decreased drastically in size (they are now up to one millionth of their original size), scientists can then employ many tiny "laboratories" on this chip. These silicon chips can analyze many different things all at once e.g. blood samples, tissues etc, and from this information, we are able to establish a diagnosis for the disease.

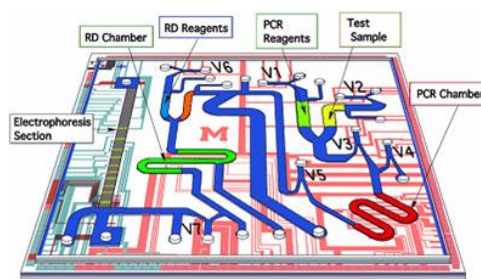


Figure 1 – Lab on a Chip

In the last few years, this idea of "Lab on a Chip" has progressed in a number of different ways. For example, Ronald Larson, a Professor of Chemical Engineering, recently instigated a new research plan where the genetic code of the influenza virus in any infected patient can be interpreted instantly by a single silicon chip. Professor Larson suggested that in the future, a "network of chips could be

wirelessly connected” which would enable the different varieties of the virus to be tracked across the globe. An alternative to this could be a global bank of the strains of the virus and the genetic make-up of these different forms, so the scientific progression of discovery and capability of tracing the viruses could be communicated across the world.

Equally, another breakthrough in the nanotechnological domain entails the idea of aptamers. Aptamers bind to specific target molecules, and are made up of oligonucleic acids or peptide molecules, they have many different uses; both for research, and for treatment and diagnosis of disease. There are two types of aptamer; nucleic acid aptamers and peptide aptamers. Nucleic acid aptamers are usually engineered using Systematic Evolution of Ligands by Exponential Enrichment (SELEX), also referred to as in vitro selection, enabling them to bind to different molecules in the body. Recently, the U.S. Food and Drug Administration legalized the first aptamer-based drug, which could be used to treat age-related macular degeneration. This has now opened doors for development of aptamers right across the medical field, and many new innovations for the prevention, detection and treatment of a vast variety of diseases. Parkinson’s disease is one of the many that has the potential to be diagnosed by aptamers, particularly the RNA (Ribonucleic Acid) type of aptamer.

Currently, to assist a diagnosis of Parkinsons disease, a PET scan (Positron Emission Tomography scan) can be used. However, in the future, nanotechnology (specifically the use of aptamers) could be combined with these scans to make diagnosis more efficient, accurate and risk-free. Research has already been conducted into Parkinson’s disease, and how aptamers could be incorporated into diagnosis, but

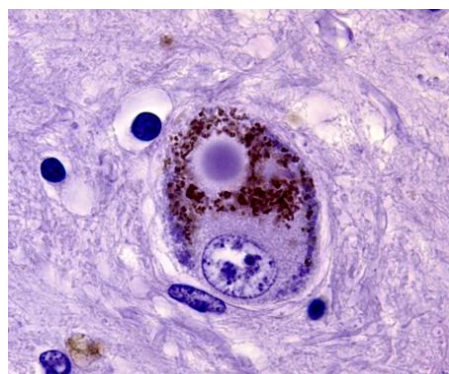


Figure 2 – A Lewy Body

the research must be developed further before the method can be implemented with patients. Alpha-synuclein has been shown to accumulate in the blood plasma of patients with Parkinson’s disease, and can lead to the formation of Lewy bodies (see figure 2), causing damage to neurons and cell death, hence interrupting dopamine pathways in the brain. This key information concerning alpha-synuclein could be used to carry out further research into new aptamers. RNA aptamers, which could combine with the alpha-synuclein in the blood plasma, would be much more effective due to their higher affinity.

It has been suggested that a reporter sequence could be attached to determine when the aptamer has bound to the alpha-synuclein in the blood plasma, however, the use of a biomarker could be just as effective, if not more accurate. Biomarkers are indicators of the biological state of the molecule they are attached to, and so if one were to be attached it would be useful, as medical professionals would be able to easily determine when the aptamer was attached. This would allow them to establish whether or not there was alpha-synuclein in the blood plasma, and therefore whether the patient had Parkinson’s disease. A possibility which could be introduced in the future, would be the use of a PET scan in conjugation with RNA aptamers, and so as the PET scan checks dopamine activity in the brain, these two could be used together to ensure that the diagnosis is much more accurate.

Once diagnosed, patients with Parkinson's face coping with the obvious difficulties of a disabling neurological condition and there is a constant struggle to keep the symptoms of the disease under control. The majority of patients are treated with the most popular pharmaceutical drug, Levodopa, which puts them at risk of developing dyskinesia (the difficulty in controlling voluntary movement, alongside discomfort of dealing with involuntary movements or motor fluctuation). Any treatment that is prescribed to help ease the symptoms of Parkinson's has to be carefully considered and it is of the utmost importance to tailor drug therapy to each patient's individual needs. Inappropriate drug prescription could have a severe impact on the patient's symptoms and functional capability.

Current Treatment of Parkinson's Disease Using Drugs

Pharmaceutical drugs have always been a vital and integral part of medicine and are used to cure or relieve symptoms of almost any disease. Currently, the symptoms of Parkinson's disease can be controlled, to some extent, by a variety of pharmacological therapies, the three most established of which are Levodopa, dopamine agonists and Monoamine Oxidase-B (MAO-B) inhibitors. However, each of these three treatments is enveloped by a number of issues. Levodopa has both the greatest symptom control and the most detrimental side effects of the three drugs; there is evidence that it may lead to increased motor complications and other adverse effects that could also be caused by dopamine agonists and MAO-B inhibitors. Nevertheless, it is almost inevitable that patients with early on-set Parkinson's disease will eventually receive some form of Levodopa as a symptomatic treatment, with guidelines stated by the Royal College of Physicians suggesting the doses are kept as low as possible. Dopamine agonists can also be used in conjunction with Levodopa as an adjuvant therapy in order to improve the medical effectiveness of the treatment in later disease or as an initial therapy in order to delay the requirement for use of Levodopa. MAO-B inhibitors may also provide symptomatic relief in the early stages of Parkinson's disease instead of Levodopa, but only provide restricted amount of symptom control. Therefore, despite numerous recommendations and guidelines for use of these drugs with regard to treatment of Parkinson's disease, there is still a tremendously large scope for improvement.

Future Drug Delivery Using Nanotechnology

Nanotechnology is being applied ingeniously to provide new, patient-friendly solutions to delivering drugs. Delivering a drug correctly, to the right part of the body, causes numerous challenges. Drugs used in the treatment of Parkinson's disease e.g. Levodopa, sometimes have limited solubility and may break up before reaching their intended destination. Drugs may also distribute unsuccessfully or inadvertently cause damage to healthy tissues. Another such problem of drug delivery is overcoming the blood-brain barrier for neurologically-targeted drugs. Many factors that affect drug delivery are commonly linked to metabolic and biochemical reactions close to nanoscale. Thus the focus on how nanotechnology may be used to overcome these problems is increasing. Researchers are looking into how nanotechnology could maintain drug levels within the therapeutics range, achieve effective targeting to the intended site of drug delivery, carry out slow release and decrease toxicity and side effects of drugs.

In the future, systems of drug delivery may be based on tiny structures that are engineered nanotechnologically, where the drug can be dissolved, absorbed or

dispersed in the matrix of the nanoparticle. A possible alternative to this would be a vesicular system in which the drug is carried in an aqueous or lipid carrier within the walls of a hollow particle. Future nano-drug delivery systems may utilise external energy sources such as ultrasound, light, or magnetic fields, all of which enable activation or release of the drug at the target site. Fullerenes are carbon isoforms arranged in spherical cage-like structures of size range 0.7-1.5nm.

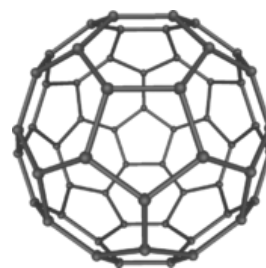


Figure 3 – A Buckyball

The first to be discovered was buckminsterfullerene, also known as a “buckyball”, by Curl, Smalley and Kroto in 1985; it consisted of 60 carbon atoms.

Alternatively, by using tubes rather than spheres, ‘smart’ bio-nanotubes of tubulin coated with lipids could encapsulate a drug (for example Levodopa), and use the electrical charges of different cellular structures to “open” the nanotubes, releasing the drug, at the desired site within the body. Nanodiamonds are also an area of active research in drug delivery, due to their large surface area and tendency to cluster. Drugs could be attached to the surfaces of individual nanodiamonds, remaining inactive while the nanodiamonds are clustered together. Then once these particles reach the intended site of delivery, the clusters break apart and release the drug load.

There are significant advantages to using nanostructures as drug delivery vehicles opposed to more traditional delivery mechanisms. These advantages include; high stability, the possibility of transporting both hydrophilic and hydrophobic drugs, high carrying capacity due to greatly increased surface area, better bioavailability, systems that allow controlled release rates or release upon an external stimulus and the possibility to exploit a range of patient-friendly delivery routes.

Treatment of Parkinson’s Disease Using Gene Therapy

There is still a great amount of potential for development of the treatment for Parkinson’s disease. This has been duly identified and is now beginning to be addressed more extensively, however rather than mere conventional drug research; investigation into treatment of Parkinson’s disease has branched into the science of nanotechnology, specifically with regard to gene therapy. Gene therapy for Parkinson’s disease involves the administration of standard, “healthy” genes into a Parkinsonian patient with defective, “faulty” genes with the aim to trigger the cells within these genes to begin producing dopamine. It is anticipated that the healthy genes begin to adapt the malfunctioning cells by making alterations to them that trigger the production of dopamine within them to resume. If this process can be achieved effectively with little or no complications, then this treatment could be a valid way of controlling or ceasing the symptoms of Parkinson’s disease.

Currently, there are a number of different approaches to the treatment of Parkinson’s disease using gene therapy, with the main differences concerning the class of product used. The two chief classes of products used are neurotrophic factors (factors that help to prevent the death of neurons) and proteins that work to amplify dopamine production. Neurotrophic factors, including Glial Cell Derived Neurotrophic Factors (GDNFs), encourage the neurons to grow and thrive and can prevent further damage being caused to these cells. In the past, scientists have endeavored to directly deliver this genetic material into the areas of the brain that require them for development and repair. However, trials undertaken have proven

this method to be impractical as it is too difficult to maintain the correct levels of the molecules in the correct regions of the brain. Considerable side effects were noted, decreasing the suitability of this method with regard to treating Parkinson's disease. An alternative to the use of neurotrophic factors is the introduction of proteins that increase the levels of dopamine produced within the brain – specifically enzymes including dopa decarboxylase and tyrosine hydroxylase. These proteins would act as an alternative to Levodopa, dopamine agonists and MAO-B inhibitors. Instead of being delivered by injection, which would simply mean that the proteins were unable to enter the necessary cells due their inability to penetrate the cell walls, the gene therapy technique allows the proteins to be delivered directly into the cells themselves. This would effectively create a new dopamine production centre within the brain – a revolutionary step concerning the treatment of Parkinson's disease.

In order to overcome the obstacles encountered when attempting to deliver certain genetic material directly to the centres within the brain that require it, new methods have been developed involving virus vectors. Before being used to carry genes around the body, the harmful, disease causing properties of the viruses are removed to ensure that they can safely transport the genetic material. Genes that encode either the neurotrophic factors or dopamine manufacturing proteins are inserted into the viruses. The recombinant viruses enveloping the genes are injected directly into the defective areas of the brain and are able transport the therapeutic material to the sites within the brain where it is needed. However, the recombinant virus vectors are at high risk of being recognised as foreign material by the body and therefore being subjected to an immune response. It has also been discovered that mutagenesis of the virus vectors may lead to the formation of tumours during carcinogenesis and even death.

Recent developments by University of Kentucky researcher David Yurek have seen a new approach to the gene therapeutic treatments. His proposed technique involved condensing DNA plasmids into nanoparticles, enabling them to be delivered to the brain as a means of ceasing or preventing the degeneration of the nervous system. This technique is non viral and therefore the issues highlighted during trialing of viral vectors are less likely to occur using this method. Yurek's technique involves using polycations to compact individual molecules of plasmid DNA to form compacted "DNA Nanoparticles" (DNPs) (see figure 4). In theory, these particles can then be injected directly into the brain of patients with Parkinson's disease as an alternative to use recombinant viruses. In addition to avoiding immune response and subsequent disastrous side effects, using DNPs instead of alternative methods ensures that even if the DNPs are injected into the targeted area of the brain, rather than inside specific cells, they are able to effectively penetrate and subsequently enter the correct area cell. This can be achieved because unlike hydrated DNA, the DNA plasmids, once compacted, are small enough to cross the nuclear membrane pore of post-mitotic cells once they have passed through the cell wall and the cytoplasm. Studies undertaken by Yurek involved injecting compacted DNPs directly into the brains in order to carry GDNF

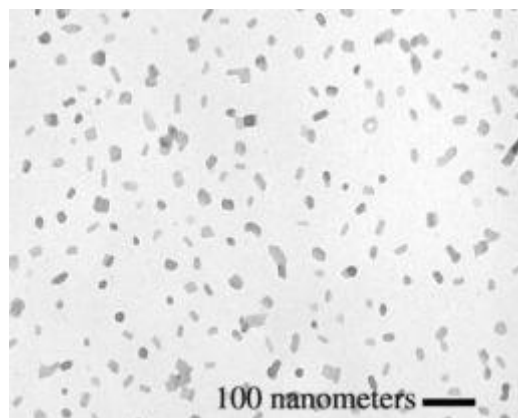


Figure 4 – DNA Nanoparticles

protein to the sites that required it as an alternative to the use of virus vectors. Results confirmed that immune activity within the site of injection was negligible and this interference was found to be as a result of the injection process rather than the DNPs themselves. This minimal immunogenic response to the injection DNPs suggests that nanotechnology could be used to create an effective non-viral alternative to overcome the issues presented by virus vectors.

Conclusion

In conclusion, nanotechnology could resolve many of the underlying problems within medicine today and improve a great number of current methods regarding diagnosis, prevention and treatment. Specifically, these developments could be applied to Parkinson's disease. At present little action can be taken to slow up the progress of the disease, so any developments instigated by nanotechnology are likely to be seen as radical. Despite this, nanotechnology poses a number of issues and a degree of risk. It is important that these risks are addressed, especially when the technology will be used within such a vast field as medicine.

At present, the "Lab on a Chip" procedure cannot be used for Parkinson's disease, even though it can be a very effective for other diseases. In the future however, the "Lab on a Chip" design could be adapted to work for Parkinson's disease – the foundations of the aptamer procedure could be used on a chip which could detect alpha-synuclein in the blood plasma, from a drop of blood on the chip. This could be very effective, but on the contrary, the research into this method would require significant funding.

Even more so than diagnosis, the prospect of using nanotechnology for the treatment of disease, particularly delivering drugs to specific sites within the body, is a very real one. It is likely that within the next few years more research will have been undertaken and progress will have been made concerning treatment of disease. Regarding Parkinson's disease exclusively, carbon nanotubes and other nano structures, could be used to deliver treatment drugs such as Levodopa and dopamine agonists. No risks have been discovered as of yet, regarding in vitro use of nanoparticles in diagnosis, however there is some apprehension about possible toxic effects when using nanoparticles for therapeutic purposes. Environmental contamination from manufacturing nanomedical appliances also poses a great risk.

Another use of nanotechnology regarding delivery of treatments is the use of DNPs instead of virus vectors in order to deliver therapeutic genetic material to designated areas of the cell. This therefore virtually overcomes the major issue of rejection of the new genetic material by the immune system and with further development could eventually be implemented on a large scale into patients with Parkinson's disease. However, a lot more research needs to be done in order to evaluate the effects of this treatment as currently, experiments have only been undertaken on rodents and the effect that gene therapy with DNPs has on humans may be substantially different.

Finally, if a set of ethical guidelines are produced then it would allow nanotechnology to be used within medicine safely and people could benefit from its attributes. In the future, if these ethical issues are resolved and nanomedicine is explored further, it could have a profound impact on the management of Parkinson's disease, and the future of medicine itself.

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