

The Use of Dendrimers in Nanotechnology and Gene
Therapy to Target Defective Genes as a Potential
Treatment for Cancer and Genetic Diseases and the
Ethics of Nanotechnology Research

BY

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ABSTRACT

In the 1980's, K. Eric. Drexler revolutionised the idea of machines, by introducing the idea of building them on a scale of molecules – nanometer scale technology. Nanotechnology since then has progressed rapidly and is now being incorporated in every field of life from the food and packaging industry to its own field of nano-medicine. According to the Cancer Research UK 1 in 500 children will get cancer by the age of fourteen. 11.1% of pediatric hospital admissions are for children with genetic disorders and 18.5% are children with other congenital malformations (Canadian Association Medical Journal 108). Advances in gene therapy with the aid of nanotechnology holds great promise for the treatment of human diseases.

The aim of this study is to investigate how nanotechnology could aid gene therapy in overcoming its current limitations in correcting defective genes through the use of nano-sized structures known as dendrimers which has could lead to future breakthroughs in the prevention of genetic diseases as well as cancer. This paper will then look at the responses to the social and ethical issues of nanotechnology research from different groups of people.

Introduction to Gene Therapy

Before discussing how dendrimer in nanotechnology are used to aid gene therapy in overcoming some its current restrictions which is the main focus of this research it is important to have a thorough understanding of the conventional mechanism of gene therapy and the limitations of this procedure in the prevention of genetics diseases.

With there being over 6,000 known genetic diseases, it comes to stand that out of the four million babies born each year 3 – 4% will be born with a genetic disease or a major birth defect ¹. Genetic diseases have had a long and famous history, known to be prevalent even in royal British bloodline with Queen Victoria being a carrier of the defective gene responsible for haemophilia and through her it transmitted to the royal families of Russia, Spain and Prussia. Genetic diseases are caused by either a missing or an abnormal gene. At times it is only the defective genes which are responsible for these disorders and other times it is compounded by environmental factors. Monogenic diseases are those which can be attributed by an anomaly in one gene such as cystic fibrosis. There are other diseases such as cancer or dyslexia which are a result of several genes interacting with environmental factors particularly the diet and lifestyle and these genes are identified as susceptibility diseases. Researchers have claimed that we all carry several such genes. In October 1990, an international team of scientists officially began the Human Genome Project in order to map the entire human genome to show where genes are in relation to one another along the chromosomes and sequencing the entire human DNA, the first rough map of which was completed on June 26, 2000 ³. The ultimate goal was to use this information to treat genetic diseases after having identified the genes which where the root of the cause of the disease development. This is an area which has had a staggering amount of funding of \$786 million by institutions like the Sanger Institute and the National Institutes of Health and Department of Energy for it to reach its final stage of completion ³.

Contrary to common belief, not all defective genes necessarily produce detrimental effects since the environment in which the gene operates is also important. An example of where a defective gene has had a beneficial effect on survival is illustrated by the relationship between sickle cell anaemia and malaria. Only those with two copies of the sickle cell gene, which produces a defective blood protein, suffer from the disease. Those with one sickle cell gene and one normal gene are carriers but not affected. These carriers of the sickle cell gene now more importantly are able to resist infection by malarial parasites. The obvious advantage of this one defective gene which makes the carrier resistant to malaria, explains the widespread presence of this gene in the populations of those areas in the world where malaria

is an endemic. However, it still stands that most defective genes cause serious genetic diseases which threaten if not the survival but the quality of life of different individuals to varying extents depending upon the genetic disease. Therefore, research into the minimising the effects if not preventing the disease itself has been carried and this has opened doors to a whole new aspect of modern medicine known as gene therapy.

Gene Therapy Used as a Cure for certain Genetic Diseases

Gene therapy is described as the technique whereby a segment of nucleotide sequence or gene is inserted into an individual cell in order for the faulty or absent gene to be replaced by a working gene, an absence of which causes a particular enzyme to be either absent or ineffective in catalysing a particular reaction. By replacing the faulty gene the body is able to correct the enzyme and hence eliminate the cause of the disease.

Several approaches are taken to correct the defective genes responsible for disease development:

- The most common approach is whereby a normal gene is inserted into a nonspecific location within the genome to replace a non-functional gene.
- The abnormal gene could also be swapped for a normal gene through homologous recombination.
- The abnormal gene could be repaired through selective reverse mutation which is essentially the mutation of an existing mutant gene which restores it to its original form.
- The regulation of a particular gene (the degree to which a gene is turned on or off) could be altered to minimise the effects of the defective gene.

Gene therapy works by a carrier molecule called a vector which needs to be used to deliver the therapeutic gene to the patient's target cells. The most common vector currently is seen to be a virus which is genetically altered to carry normal human DNA. These viruses have evolved a way of encapsulating and delivering their genes to human cells, almost in pathogenic manner. Scientists have tried to take advantage of this capability and manipulate the virus genome to remove the disease causing genes and insert therapeutic genes. Target cells such as the patient's liver or lung cells are infected with the viral vector. The vector then unloads its genetic material containing the therapeutic human gene into the target cell. The generation of a functional protein product from the therapeutic gene restores the target cell to a normal state.

Different types of viruses are used as gene therapy vectors:

Retroviruses which are a class of viruses can create double-stranded DNA copies of their RNA genomes.

Adenoviruses are a class of viruses with double-stranded DNA genomes that cause respiratory, intestinal, and eye infections in humans. **Adeno-associated viruses** are another class of small, single-stranded

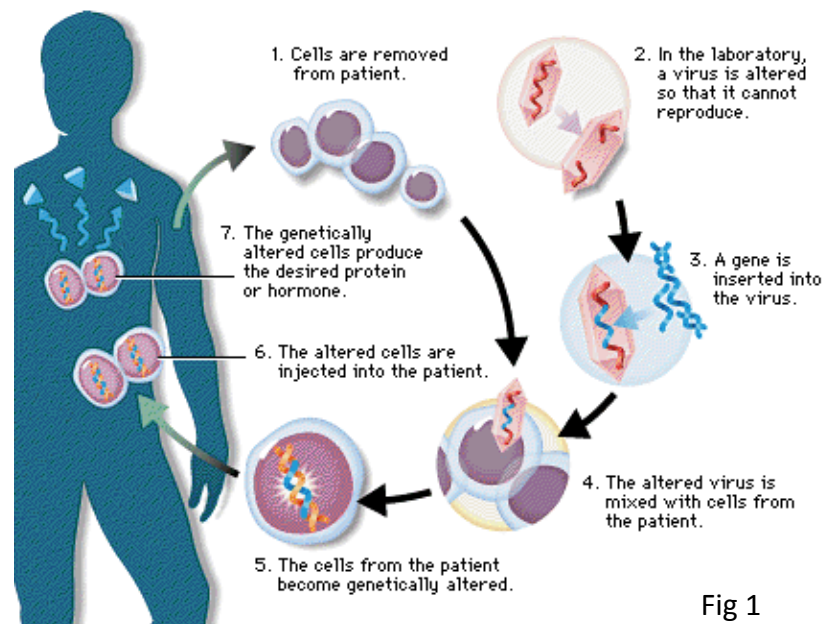


Fig 1

DNA viruses that can insert their genetic material at a specific site on chromosome 19.

Herpes simplex viruses finally are a class of double-stranded DNA viruses that infect a particular cell type, neurons. Herpes simplex virus type 1 is a common human pathogen that causes cold sores.

During a recent development, researchers had also been experimenting with introducing a 47th (artificial human) chromosome into target cells. This chromosome would exist autonomously alongside the standard 46, which would not affect their workings or their or causing any mutations.

Gene therapy has however had some setbacks. In January 2003 the Food and Drug Administration (FDA) temporarily halted all gene therapy trials using retroviral vectors in blood stem cells after they learnt that a second child treated in a French gene therapy trail had developed a leukaemia like condition. Both this child and another that had been treated in August 2002 had been successfully treated by gene therapy for X-linked severe combined immunodeficiency disease (X-SCID) also known as the 'blue baby syndrome'. In addition to this there has been other problems arisen from gene therapy which includes the risk of automatically stimulating the immune response of the body by the entry of a foreign invader which always turns to be a potential risk. Finally there are also the issue that viral vectors present a variety of potential problems in patients listed as toxicity, immune and inflammatory responses, and gene control and targeting issues. It is here that nanotechnology can now be used to overcome some of these limitations which are stated above.

Nanotechnology Aids Gene Therapy

Nanotechnology is a scientific field which is undergoing a rapidly progressive development. The roots of nanotechnology can be traced back to groundbreaking developments in not only medicine but also robotics and genomics. When E. Drexler originated molecular nanotechnology concepts at MIT he came up with the idea of a sea of minuscule robots that could move molecules at rapid speeds. In the bloodstream these robots could cure diseases. His vision inspired many scientists and researchers to focus on science at the nanoscale. It was soon after in 1985 that Buckminster fullerene or C60 molecules were discovered by Professor Sir Harry Kroto, and two Rice University professors, chemists Dr. Richard E. Smalley and Dr. Robert F. Curl Jr. and is the only molecule composed of a single element to form a hollow spheroid which gives the potential for filling it, and using it for novel drug-delivery systems. Miniaturisation of these devices at a molecular level means cost effectiveness and more rapidly mechanical, functional and biological components. In addition to this, research with nanotechnology in medicine has shown that these devices exhibit such unique self ordering and assembly behaviours both of which set them apart from macro objects. Nanomedicine has seen the development of nanobots which are used to perform the smallest of function both inside and outside the body. In order for these nanobots to function, nanocomputers are also built alongside them to aid them, and as a result quantum computing has also developed. Due to the construction of such small structures, we would for the first time, in essence, be able to send a miniature 'robot' into the cell that could be controlled externally. The possibilities of how we could use this to our advantage are endless and will be discussed further in relation to its usefulness for genetics diseases and cancer in the following section.

Discussion

Dendrimers in Nanotechnology used to aid Gene Therapy in the Treatment of Cancer

It has been estimated that there were 400,000 more cancer cases in 2006 than there were in 2004 and 1.7 million deaths from the disease in the whole of Europe. (9) Thus with such disturbing consequences it has become even more crucial to find a cure or a method of prevention of spread and killing of cancerous cells in the body. The problem however remains that with so many different types of cancer they all act differently and are different to control. It is due to this that it is so difficult to find a single effective treatment. The progress and development of nanotechnology may however provide some solutions to a possible treatment of cancer.

Nanotechnology, being in its initial research stages had been unable to take an active role in medicine until recently. As a result of the progressive advances being made, was able to destroy cancerous cells with a highly targeted pack of 'tumour bursting gene' as stated in the BBC article of 10th March, 2009. This ensured the safety of noncancerous cells and now gave hope to those types of cancer where surgery was not possible. Dr. Andreas of Schatzlein from the School of Pharmacy, London states that "*Gene therapy is an exciting area of research, but targeting genetic changes to cancer cells has been a major challenge. This is the first time a solution has been proposed, so it's exciting news.*" The biggest challenge for cancer therapy had been the prime issue of selectivity. Many of the commonly used chemotherapy drugs such as cisplatin and docetaxel aren't selective and thus result in distressing side effects for the patients like thinning of the hair, nausea and vomiting for 24 hours or longer and kidney damage to name just a few. Modern 'targeted' treatments such as Hereceptin and Tarceva were introduced which locked onto the surface of cancer cells to increase their selectivity. However the search for a treatment that exclusively targeted cancer cells whilst leaving the normal body cells unharmed still seemed to be just a possibility until the research carried out by the Cancer Research UK scientists that based their treatment on nanotechnology to target cancer cells. Andreas of Schatzlein and his team at the London School of Pharmacy had been investigating chain like molecules called dendrimers for many years. When dendrimers are mixed with DNA molecules or cancer drugs, they form microscopic balls, nano particles. Dendrimers form one layer at a time so the size of the dendrimer is determined by the number of synthetic steps. A dendrimer is only a few nanometers wide. The outside layer can be engineered to be composed of specific functional groups that can act as hooks to specifically bind other molecules such as DNA. Due to the utility of the structure of these dendrimers I believe that they are the future of cancer treatment. On the above based research, it seems highly probably that dendrimers would be successful in acting as affective agents for delivering DNA into cells during gene therapy.

In 2006 Swiss scientists stated that 'gene transfection, the delivery of genetic material into a cell is currently most efficient using viral vectors'. The success of the viral gene carriers was partly attributed to their self assembly as this improved their passage through the cell membrane. With this in mind the Swiss team incorporated lipophilic branches into the dendrimer. This lipophilic section was linked by an aromatic group to cationic branches involved in cellular uptake. (10)

The structure of the two branched sections and the linking group were varied to determine their influence on the biological activity of the molecules in human cancer cell.

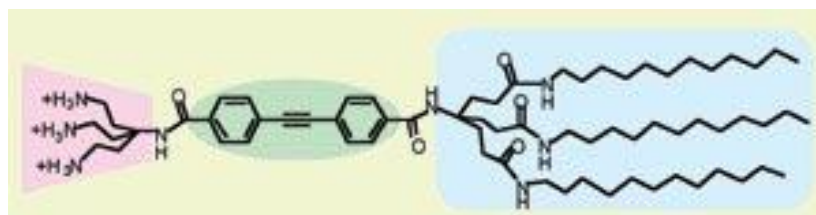


Fig 2

It was seen that there was a positive correlation between the self assembly and the transfection efficiency of the dendrimers. It was also noted that the nature of the lipophilic branches were found to be an important factor for the self assembly of the molecules for the transfection efficiency.

The results of mixing DNA drugs and dendrimers were such that the created nanoparticles were highly attracted to the tumours that was responsible for fuelling cancer. Due to their tendency of building up in tumours, Schatzlein and his colleagues were interested in using them for gene therapy. They wanted to transport specific genes into cancer cells causing them to make toxic proteins that can kill cells whilst leaving the surrounding tissue unharmed. In 2005 they experimented this idea on mice by using dendrimer particles to transport the gene carrying instructions for a toxic protein called ‘tumour necrosis factor alpha (TNF α) into cancer cells in mice. The results were highly successful – the tumours shrank and the mice survived significantly longer. This experiment proves the alternative proposition that this paper makes that instead of using chemotherapy which has distressing side effects for the patients, it seems more feasible to deliver gene therapy to tumours around the body. Future treatments based on this technology could use nanoparticles that combined a tumour killing gene with an iodine reporter gene and through CT scanning (which detects radioactive molecules), it would be possible to build up an impressively detailed picture of the location of the tumors throughout the bodies of the mice. The dendrimers which would be specifically targeted to attacking only the harmful cancerous cells would not produce the side effects that current chemotherapy drugs do.

Dendrimers Used to Correct Defective Genes as a possible treatment of Genetic Diseases

Now that this study has shown with evidence of experiments conducted out by the team at the London School of Pharmacy, it can also be deduced that these dendrimers which have been so successful in carrying genes for toxic proteins to the cancerous cells, could also be in fact used to treat many genetic diseases. These nanostructured synthetic molecules could in theory be carriers of a gene which could potentially replace the defective gene in a cell, hence correcting the genetic deficiency. Where viral vectors had the problems of rejection, immune and inflammatory responses (as mentioned above) in conventional forms of gene therapy in principle, dendrimers should not have a similar effect on the body. Safety is a primary concern when working with gene therapy. Viral vectors have been found to be dangerously toxic and this was tragically demonstrated when an 18-year old boy enrolled in a gene therapy study had a massive immune reaction to the virus used resulting in death in only a few days due to multiple organ failure. Once perfected gene therapy could save the lives and improve the quality of lives of millions but there still seems to be many problems to overcome in gene therapy. This study proposes the use of dendrimers as a possible solution due to the following reasons. Dendrimers are much less toxic and they offer other advantages in terms of cost, ease of production and the ability to transport very long genes. They can be designed to efficiently and safely shuttle genes into human cells. It is due to this that I propose that dendrimers would be a more practical solution to gene therapy.

These nano-sized structures may well become the long searched Holy Grail for the effective treatment of cancer. There is still a long way to go until we could start the clinical trials of this nano-treatment in patients since the safety of giving this treatment of patients still needs to be looked into and worked out. Although the results of using dendrimers are encouraging there are still relatively early experiments using animals and so it is possible there we are a few years away from clinical trials of this nano-treatment in patients.

The Social and Ethical Issues of Nanotechnology:

In this research, I have shown just how useful and essential nano-sized structures could become to the medical health sector in helping to combat prevalent genetic diseases as well as forms of cancer. However, there are certain implications of nanotechnology which seem inevitable if research and development into nano sized structures continued. Firstly, these assembler and disassemblers could be used to create weapons or used as weapons themselves. Miniature weapons and explosives would be a perilous result of the progressive development of nanotechnology since these miniature weapons would prove to be more deadly and easier to hide and explode when time needed. With nanotechnology, armies could also develop disassemblers to attack physical structures or even biological organisms at the molecular level. A similar hazard could occur when general purpose disassemblers got loose in the environment and started disassembling every molecule they encountered, something known as 'The Grey Goo Scenario'. Furthermore, if nanomachines were created to be self replicating and there was a problem with their limiting mechanism they would multiply endlessly like viruses. This hypothetical situation might show nano technology as more powerful or prolific than it may ever become, but there are still many other ethical concerns with the use on nanotechnology.

Whilst conducting nanotechnology research it is essential to seriously consider the impact on the health and well being of fellow researchers reporting on any unsafe practices. The environmental consequences of the research also need to be considered i.e. the minimisation and safe disposal of hazardous substances. The bad news here is that scientists have no way to track nanomaterial like bucky balls or nanotubes in the environment. Even if they were to be found there would be no way of removing them from the soil or the water. In addition to this scientist as such have no way of acceptably finding them or removing them from the human body. There has also been a lack of studies of the effect of nanotubes, bucky balls, or other nano materials on the human physiology or the environment. There is a possible danger that because nanomaterial are smaller than cells, they might enter cells or bio accumulate in smaller creatures and in this way would be able to work up the food chain in ever increasing concentrations until they start to cause problems for humans. Not only would environmentalists be opposed to such research where there could be potential harmful effects to the environments which are still unknown but if nanotechnology was to be introduced in the food industry, many consumers would be weary unsure of the effect of the nanotechnology to their human physiology.

Conclusion:

As a result of the research conducted in this paper, it is clear that although there is still a long way to go; nanotechnology has become and will soon become an even greater part of medicine. This paper supports the use of nano technology particularly dendrimers, recognising its importance in the future of gene therapy, whilst at the same time acknowledging the issues that surround research that involves nanotechnology. However, it does stand true that there are also many ways around these issues which would include further testing to determine the impact of nanowaste on the environment and the human physiology, methods of detecting nano waste and finding a suitable way of disposing the nanowaste.

Nanotechnology has been one of the greatest developments of the 21st century and advancements in it has led to revolutionary leads in other areas of medicine, particularly gene therapy. Where previously it had been difficult to transfect DNA or genes into a cell using viral vectors without some kind of immune/inflammatory response from the body, it is now possible to use dendrimers which are not only more cost effective but also do not produce any distressing side effects which are common in patients that undergo gene therapy. Curing genetic diseases such as sickle-cell anaemia and cystic fibrosis now seem like an advancement of the very near future.

Gene therapy has also been supported by the Reverend Russell Smith, president of the Pope John Medical-Moral Research and Education Centre, stated that gene therapy is "a very noble enterprise, because it is aimed at the actual cure of actual diseases." Gene therapy targets cancerous cells but is very specific in the sense that it leaves the body cells unharmed without producing any of the usual side effects of the chemotherapy drugs. Once introduced, it could be predicted to be a very popular choice over chemotherapy.

Nanotechnology in relation to gene therapy does however have some drawbacks in itself. The success of the results of experiments in mice although are encouraging does not provide us with a conclusive set results as of yet on the realistic effectiveness of this new treatment using dendrimers. The conclusion could only truly be made once there has been some human testing. Based on the outcome of the trials, dendrimers as predicted by this research project could revolutionise nanomedicine, saving and improving the lives of millions that suffer from genetics diseases or different forms of cancer.

References:

- (1) 'Statistics on Genetic Diseases'
Nussbaum, RL, McInnes RR, Willard HF. Thompson & Thompson's Genetics in Medicine, 7th ed. 2007, WB Saunders Company, Philadelphia, PA
- (2) http://www.ornl.gov/sci/techresources/Human_Genome/home.shtml
- (3) 'The Scientists say Human Genome is Complete – by NICHOLAS WADE '
- (4) BIO. "Biotechnology in Perspective." Washington, D.C.: Biotechnology Industry Organization, 1990
- (5) 'What is human gene therapy' - By Ningthoujam Sandhyarani
- (6) 25 Ways Nanotechnology is Revolutionizing Medicine - January 19, 2010 Medica'
- (7) 'Structure of a New Family of Buckyballs Created' – June 2009
- (8) <http://scienceblog.cancerresearchuk.org/2009/03/24/nano-scale-advance/>
- (9) www.bio-medicine.org
- (10) 'Gene Delivery with dendrimers' M Guillot *et al.*, *Org. Biomol. Chem.*, 2006,
- (11) http://www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml
- (12) <http://news.bbc.co.uk/1/hi/health/7935592.stm>
- (13) http://www.greatnewsnetwork.org/index.php/news/article/nano_treatment_to_torpedo_cancer/
- (14) <http://www.molecular-plant-biotechnology.info/animal-biotechnology-genomics/biotechnology-medicine-gene-therapy/nanotechnology-for-drug-targeting-and-gene-therapy.html>
- (15) <http://www.biotecharticles.com/Nanotechnology-Article/Application-of-Nanotechnology-in-Medicine-216.htm>

Image References:

Fig 1 –Procedure of Gene Therapy - *Microsoft Encarta Online Concise Encyclopedia*

Fig 2- Gene delivery with dendrimers 24th February 2006