How can nanoparticles aid with gene and drug delivery?

By

Puja Khajuria

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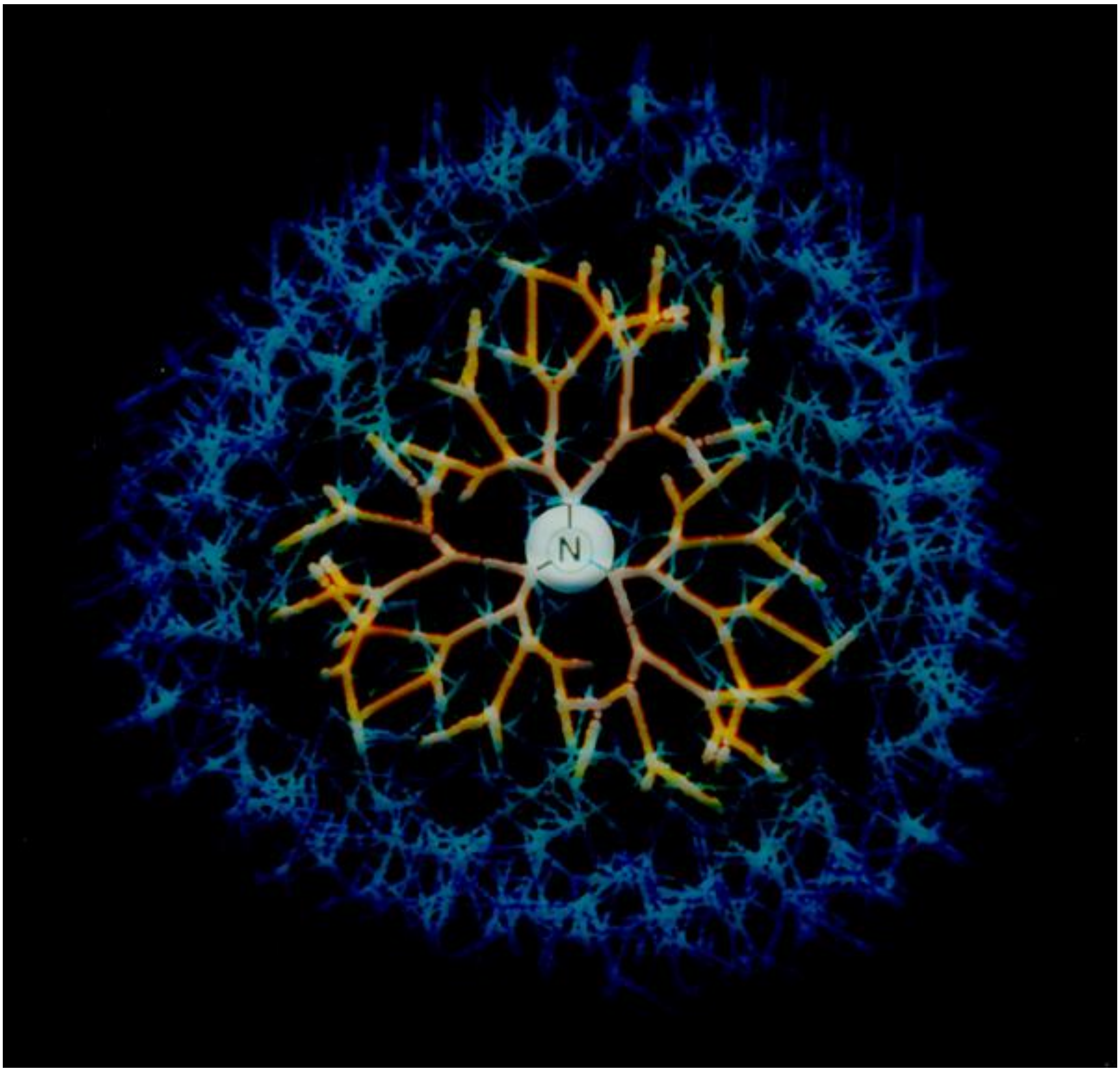


Research paper based on Pathology lectures at MEDLINK 2010

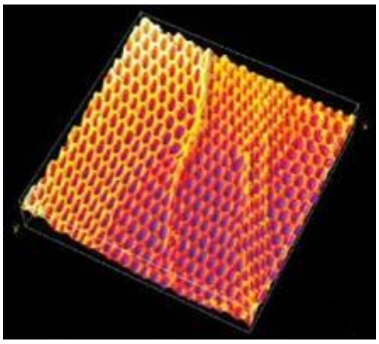
Abstract

Nanotechnology is newly discovered aspect of science that has infinite capabilities of changing our current technology to be more efficient, accurate and safe. It is still far from being introduced into societies however its many uses are currently being researched and tested. I will be focusing on the effect nanotechnology could potentially have on genetic disorders, and its use in gene therapy. I will discuss the use of tools used in gene therapy aiding in drug delivery, as well as investigating the effect nanoparticles could have on the blood brain barrier, so that nanotechnology can be used to treat neurological disorders such as Alzheimer's by delivering therapeutic agents directly to the brain.

Introduction

Nanotechnology explores the prospective applications and capabilities of particles smaller than 100 nanometres. What makes nanotechnology such an important development in the medical realm is the fact that it can manipulate our internal workings which in itself are so small. For example cells which are the building blocks of organisms allowing us to survive, and are like nature's nanomachines producing vital molecules. Technology of the nanoscale dimensions can function at the cellular level and perhaps contribute to the effect cells have on an entire organism. Nanoparticles are small enough to pass through our vessels, and even cross the blood-brain- barrier in human beings.

There is a great amount of research going into nanotechnology. Currently scientists have regenerated nerve cells in the spinal cord of rats. When nerve cells in the spinal cord are damaged perhaps by an accident they cannot heal and can cause permanent disability. "Rats with spinal cord injuries recovered mobility in their hind legs, raising hope that the approach might one day help people with paraplegia" [1] says Andy Coghlan from the new scientist. The Italian researchers who carried out this experiment developed "nano-conduits" [1] which are tubes that aid in guiding the formation of nerve cells. One of the factors preventing nerve cell regeneration are fluid filled cysts that form at the site of injury, however this obstacle can be overcome by these tubes guiding the nerve cells to re-grow. However the application on humans is still in the distant future as the procedures side effects have not yet been investigated.



Some very new research currently taking place involves using nanotechnology to help grow organs and help wounds heal using fabric which is 10 nanometres thick and is made from the proteins present in muscle tissue. Kevin Kit Parker's team from Harvard University made this fabric

Figure 1

(figure 1) that “provides a scaffold” [2] which guide cell growth, thus giving the tissue to be formed the correct structure. The research team created heart muscle cells which grew on this specialized fabric. However this element of research in nanotechnology is still very new, therefore experimentation on living organisms has still not been conducted. The properties and capabilities of this fabric still need to be tested before further progression into medical application.

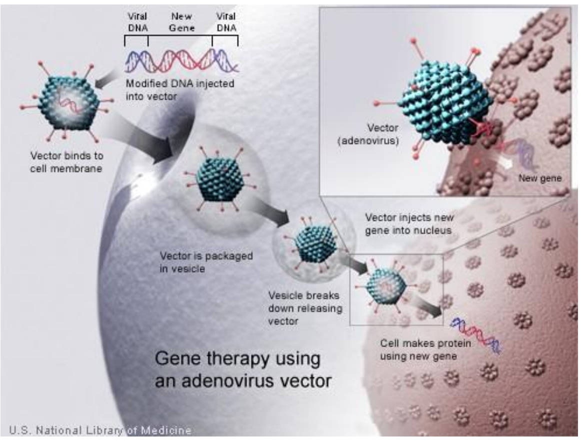


Figure 2

As you can see nanotechnology can help in carrying out procedures and processes that were once thought impossible. Due to the sheer size of these materials; they can target the route of the problem all the way down to particular cells causing the disruption, and can change the lives of people who were thought to have untreatable diseases or conditions.

The one of the areas of nanotechnology that I will be focusing on is gene therapy. There are a vast variety of different genetic disorders causing problems to a high proportion of the population, and these keep becoming problems due to gene mutations being passed down in families affecting later generations. One of the obstacles in genetic therapy is the lack of efficient and safe gene transfer to target cells by using a vector which is what transports the gene to cells. Currently viral vectors have been used in clinical trials. They are ideal because they have the ability to invade host cells and stimulate protein synthesis of the specific gene and cause the desired effect. Figure 2 demonstrates how the viral vector transports the genes. However the immune response caused by viral vectors poses as a barrier. In 2003 a trial conducted by French researchers was halted as patients being treated for an immunodeficiency disorder were developing leukaemia

This is where nanotechnology can make a difference. Nanoparticles like dendrimers, microbubbles, or nanoscale DNA films can be a non-viral means of delivering genes to target cells efficiently and safely. Some researchers working in the institute of technology used nanoparticles to deliver genes that commanded ovarian cells to die to help cure ovarian cancer. This experiment was carried out in mice and was proven to be successful. They say they “could be ready for human clinical trials in as little as a year.”[5] So this application of nanotechnology could be one of the first successful medical uses changing people's lives.

The other area of nanotechnology that I will be focussing on is its effect on drug delivery mechanisms. The way it can localize a drug to cause minimal side effects, and its ability to penetrate and breach the blood brain barrier to help those suffering neurological disorders. Current technology is cannot breach this barrier to the brain to enable therapeutic agents vital for the organ to be delivered, therefore much more complex methods have to be used. This ability can cause a great difference to treating the brain, it makes it a great deal simpler and much more efficient as the drug can be localised. It is developments like this that can make a difference to millions of civilian's lives.

Discussion

My idea for a possible future development for a medical use for nanotechnology is to use this advance in technology to help treat genetic disorders like Duchenne's muscular dystrophy. This disorder is caused by a gene mutation on the X chromosome which is responsible for the manufacture of dystrophin which is the protein which is situated under the membrane that surrounds the muscle fibres known as the sarcolemma. The gene mutation results in a lack of dystrophin in an affected patient, as much less is produced. This causes the sarcolemma to become much more vulnerable to damage, and can be damaged by the muscles contracting which causes tears in this membrane. This allows calcium to diffuse through activating enzymes that break down the proteins in the muscles causing destruction to the cells in muscles. This leads to the muscles loosing strength which will eventually cause a loss of strength and mobility. As soon as the muscles present in the heart and respiratory system are affected the condition can cause death. There is no cure for muscular dystrophy at the moment however there are treatments to help reduce the effects of the symptoms. [6]

In 2009, gene therapy on 6 monkeys thigh muscles was conducted which improved muscle strength of these primates and this experiment was carried out in order to find treatment for patients with muscle wasting disorder like Duchenne's muscular dystrophy. The gene coding for the protein follistatin was inserted into the muscle cells of these primates using the common cold virus as a vector to transport the genes. Myostatin is a protein that prevents muscle growth, which has a greater effect in adulthood when a healthy and appropriate muscle tone and mass is present, whereas in childhood it hardly has an impact.

Follistatin is a protein that blocks the action of myostatin so that muscle growth is not inhibited. This therapy was conducted on primate's legs, and was "15 per cent bigger in circumference on average after eight weeks."[6] The strength and size of these muscles remained till 15 months after the treatment and there were no health problems or negative side effects. This shows that gene therapy could be a tool to allow people with muscular dystrophy to retain their mobility and strength for a much longer time.

If nanotechnology can be used to manipulate the genes of cancer cells, what's stopping it from being used in manipulating the genes of target cells to treat duchenne's muscular dystrophy? Dendrimers shown in figure 3 are nanostructures designed to contain molecules in its core or on its surface. They are made starting from an atom, and then other elements are attached to the structure through chemical reactions which results to form a branched spherical structure which is slightly positively charged. This makes them ideal to transport genetic material as DNA is slightly negatively charged therefore an electrostatic attraction can occur between the dendrimer and DNA. The dendrimer carries genetic material coding for the normal/correct version of the gene and will enter target cells via endocytosis (figure4) which is a method of transport across the cell membrane. After entry into the cytoplasm the DNA needs to enter the nucleus to take advantage of the "nuclear transcription machinery and initiate gene expression." [7] Access to the transcription machinery can essentially occur during cell division when the nuclear envelope disappears, allowing molecules to move between the nucleus and cytoplasm. Once the genetic material has access to the tools in the nucleus the genetic material can undergo transcription and translation in order to produce the necessary proteins to help cure the genetic disorder. So in the case of muscular dystrophy the gene coding for follistatin will be delivered by dendrimers to target cells which would be

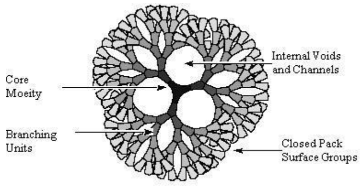


Figure 3

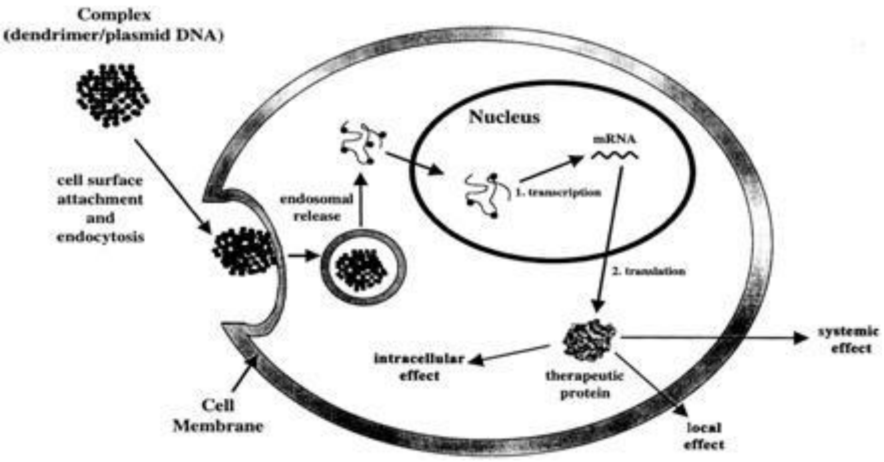


Figure 4

cells in the muscles. Once the DNA has entered the nucleus transcription of DNA into mRNA will occur in the nucleus, and then translation will occur at the ribosome's that are attached to the rough endoplasmic reticulum, as the proteins produced at these ribosome's are those that need to be excreted by the cell. The proteins made by the ribosome's then move to the rough endoplasmic reticulum and are folded and processed into their 3D structure here(sugar chains are added). After this it is transported to the Golgi apparatus by transport vesicles and here the proteins move through the sacs of the Golgi and is modified, for example carbohydrates could be attached. Then the final protein is transported out of the cell by vesicles, and these vesicles fuse with the cell surface membrane and release its contents via exocytosis. Therefore follistatin is released from the cell to the muscles, so that the muscles strengthen due to the binding of follistatin to myostatin. "Activated dendrimers can carry a larger amount of genetic material than viruses. SuperFect–DNA complexes are characterized by high stability and provide more efficient transport of DNA into the nucleus than liposomes." [8]

Another method of transport of genetic material involves nanoscale DNA films, developed by a group of engineers in the University of Wisconsin. They created ultrathin nanoscale films that are made of DNA and soluble polymers which permit the controlled release of DNA. Once they are near the target cells the films degrade and release the DNA. Also, the engineers created the films to tightly package the genes so that it can easily pass into cells. The DNA then passes into the nucleus and uses its transcription machinery to produce the necessary proteins. These films were tested as a coating for medical devices, and in one experiment it was used to coat stents. The scientists have already managed to deliver genes to cells grown in a dish and DNA film-coated stents has also managed to a gene coding for a fluorescent protein into a rabbit's artery thus showing that these tools can function in complex and harsh environments like the conditions inside a living organism. I propose that these DNA films could be used to deliver the gene coding for follistatin to muscle cells for patients with Duchene's muscular dystrophy. [4]

One of the drugs used to help reduce the symptoms of muscular dystrophy are glucocorticoids. This drug helps by slowing the process of degeneration in the muscles by increasing the strength of the muscle cells [10]. This drug is a catabolic steroid meaning it breaks down tissue which releases glucose and energy, and some scientist believe that it even triggers the manufacture of muscle protein. Nanoparticles not only have a use in gene delivery, they are also thought to be ideal for drug delivery. Nanoparticles can encapsulate the appropriate drugs and transports it directly to the tissues and cells that require it. This in turn increases the effect of the drug as well as minimizing any side effects. Previously liposome's were being investigated as drug carriers, however they proved to have low encapsulation efficiency. The advantage nanoparticles have over liposomes is that it has much better properties allowing a controlled release of drugs. Also nanoparticles have a higher surface area to volume ratio therefore this makes diffusion of drugs much more efficient. Therefore nanoparticles such as dendrimers could be used to for targeted drug delivery in patients suffering from Duchenne's muscular dystrophy.

An obstacle in treatment of the brain is in fact the blood brain barrier. It is a structure in the central nervous system made of endothelial cells guarding the blood vessels that keeps harmful substances in the blood stream away from the brain where it could potentially cause harm. This is an issue when it comes to treating with problems in the brain as certain therapeutic agents needed to treat a problem in the brain are too large to pass this barrier, and in this way nanotechnology could provide a solution to this obstacle. Nanoparticles are small enough to pass through, and nanoparticles can even encapsulate substances within them so perhaps certain vital substance can be delivered to the brain in these particles. In present day, drilling holes in the skull are required to deliver drugs to affect the brain. It is a time consuming procedure and involves direct access to the organ. This treatment localizes the drug to where it is needed as well as being a much easier means of delivering a drug to the brain, which could make those suffering with disorders like Alzheimer's or suffering from brain tumours much easier to treat. [12]

Another method of drug and gene delivery is to use microbubbles of the nanoscale size. They encapsulate the genes or drugs in the bubble and when exposed to

Figure 5

ultrasound radiation of particular amplitude, they burst and release their contents to the target cells or tissue (figure5). When the mircobubble is exposed to ultrasound radiation, it expands and contracts and releases its contents, as well as accelerating the microbubble to a vessel wall so that it is closer to target cells. Some scientist in China carried out experiments involving gene delivery with microbubbles. The gene for beta-Gal was delivered to vascular smooth muscle cells, and a variety of different microbubbles were used. Their transfection efficiencies were analysed and compared to a control variable, and gene delivery through microbubbles proved to be much more efficient, about 6 times more efficient than the control. These microbubbles have also shown to increase the membrane fluidity when they burst making cellular uptake more efficient. Ultrasound radiation in itself has shown to stimulate certain repairing genes as well as having the ability to create holes in the cell membranes of target cells, and both of these implications improve transfection. So I think that even microbubbles could be used to help treat Duchenne's muscular dystrophy. They could carry the gene coding for follistatin and deliver it to muscle cells. [11]

These microbubbles can even be used to help with the blood brain barrier issue. The microbubbles are injected into blood vessels and travel to the capillaries present in the brain. The mircobubbles have the ability to cause a reversible change to the blood brain barrier so that it is not damaged. Pulses of ultrasound radiation are introduced to the bubbles and cause the blood brain barrier to become permeable and allow the entry of the drug. This process was carried out on mice and rabbits by Kullervo Hynynen a medical biophysicist and a group of other scientists from the University of Toronto. They managed to open up the blood brain barriers in these organisms without long term damage. Another group of scientists used a similar method, firstly the bubbles were injected into the bloodstream along with a drug targeted to the brain and ultrasound radiation is used to direct them towards the blood brain barrier. The bubbles then manage to open the blood brain barrier, and then nanoparticles containing drugs are injected into the patient, and are guided to the area of the brain that require the drug most. "so far rodent studies have shown as much as a 20% increase in the amount of antitumour or Alzheimer's that reaches the brain when ultrasound and microbubbles are used"[9] reports Jeneen Interlandi from the Scientific American.

Ethical issues surrounding nanomedicine:

Although nanomedicine could potentially be a life changing aspect of medicine in the future, there are still many unknowns to this subject merely because of its recent founding. Its effects need to be investigated, such as to health, the environment, and societies.

Firstly, one of the ethical issues to discuss is the nanomaterials involved in therapies. There are a vast variety of different nanomaterials and each is as different due to its chemical properties as they are designed all the way down to the molecular scale. The only property nanomaterials share are their size, otherwise each are so different. This makes it tricky to assess the possible risks it could have to the human body. Also scientists fear that toxic particles could pass through the blood brain barrier and cause serious damage to an individual. It could cause changes in the genome and maybe generate a severe immune response.

The implementation of nanoparticles are erratic, therefore they may behave differently in an artificial environment than in a living organism. Therefore because of its random behaviour it is much more problematic when determining the risks of these particles to humans. Also the behaviour of nanoparticles in animals could be much more different than its behaviour in humans therefore the risks drawn from studies on animals can never be certain, as different organisms have different reactions to the particles.

Nanotechnology is a subject that is extremely complex and easy to misunderstand. Therefore when conducting clinical trials on humans, it would be difficult to explain the therapies involved in the trial to the participants, so an informed consent (a decision based on a full understanding of the therapy) is much harder to achieve. Also patients must be told about the lack of knowledge scientist have in consequences of treatment since long term side effects of therapies involved are unknown and unpredictable.

Quite an important ethical issue of nanomedicines and treatments is its availability to people of different financial statuses. Since it is a fairly new technology it will be extremely expensive therefore only the wealthiest of the population will be able to afford the advantages that this technology has to offer. Leaving the poor treated with much older technology, and this will create a much bigger gap between the rich and the poor causing inequality. Developing countries would not be able to afford this technology and it is those who reside in these countries that actually need the technology as they have greater health problems than those living in developed countries.

Conclusion

Overall I think nanotechnology could make a great change to lives of those people suffering from genetic disorders. Not just Duchenne's muscular dystrophy but other disorders like cystic fibrosis and especially genetic disorders affecting the brain because of the nanoparticles being able to cross the blood brain barrier.

Some studies conducted show that a limitation of using nanoparticles for gene therapy is the transport of the particles into the cell. Sometimes they stay in the vesicle and so are then transported back out of the cell via exocytosis, therefore causing a lack of efficiency. Even thought a non-viral means of gene delivery has a better response to the immune system than viruses it still has many other limitations. Non-viral vectors do not have the same skill as viral vectors in invading cells and using its machinery in to replicate and enhance gene expression. However in nanoparticles that mimic viral vectors are developed then gene therapy could be a treatment we will see much more often in the future.

The other issue of using nanoparticles is its unpredictable behaviour. Who knows what it could do it the brain? Who know how it will react in the body? These are questions that be answered before nanotechnology could ever have an impact in medicine. Will it be a development that saves lives? Or ends them? Much more research, experiments, and studies needs to take place before any of these questions are answered. Perhaps in the future scientist may develop a method of controlling the erratic nature of these particles to make a huge difference to mankind.

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Images:
Figure 1: [2]
Figure 2: wiki, gene therapy, http://psychology.wikia.com/wiki/Gene_therapy
Figure 3: [8]
Figure 4: [8]
Figure 5: [11]