

**CROSSING THE BLOOD BRAIN BARRIER WITH
NANOTECHNOLOGY**

BY ELIZABETH HOUGHTON

PASS WITH MERIT

**RESEARCH PAPER
BASED ON
PATHOLOGY LECTURES
AT MEDISIX 2011**

Abstract

The Blood Brain Barrier is arguably the biggest obstacle in the treatment of neurological diseases. This highly selective anatomical barrier is one of the many protection mechanisms of our most delicate organ, and although it is highly effective for the most part in preventing potentially harmful micro-organisms and toxins from entering the brain, in the diagnosis and treatment of many neurological diseases it severely reduces not only the treatment options available but the effectiveness and efficiency of the treatment. With the Blood Brain Barrier now being more thoroughly understood and the current developments along with the potential developments in nanotechnology being recognized, it may be possible that nanotechnology will revolutionize the treatment of neurological diseases by creating an effective delivery system over the Blood Brain Barrier. In this paper I will investigate the possible mechanisms of achieving this and also highlight some of the limitations of my ideas.

Introduction

Nanotechnology is the study of manipulating matter at the atomic scale, and since the speech of Richard Feynman in 1959 entitled 'There's plenty of room at the bottom,' the developments in nanotechnology have become a prevalent part of scientific research. The term nano, Greek for dwarf describes a scale of 10^{-9} metres. Put in context one nanometre is one millionth of a millimetre. The principle of nanotechnology lies not on the basis of taking away from a device to make it smaller but starting on the smallest possible scale, and through manipulation, addition of particles and the mimicking of nature, create a product with a specific and highly adapted function.

The prospect of the use of nanotechnology in medicine is vast and the NIH (National Institute of Health) currently in the second phase of its nanomedicine program, say the goal of the common fund of nanomedicine is to "determine how cellular machines operate on the nano level and then use these design principles to develop and engineer new technologies and devices for repairing tissue or preventing and curing disease."(NIH, 2005). The program has since been researching the chemical and physical properties of materials and biological structures of the nanoscale. The prospects for use of nanotechnology in medicine is so great due to the relative size of the nanoparticles in comparison to the cells making up the human body. Many aspects of medicine rely on the understanding of cellular mechanisms and functions, and nanotechnology allows an extensive investigation into diagnosis and treatment on the smallest level.

The great interest into the development of nanoparticles is built on the basis that materials at the nano level bear different physical properties to the same material when it is used on the macro level. For example the smaller nanoparticles have a higher surface area to volume ratio than molecules with larger dimensions, this is a useful property if a nanoparticle was to act as a catalyst on which chemical reactions can occur. For example, buckminsterfullerene is a hollow structure with chemical formula C_{60} , it was first discovered in 1985, and its discovery won Robert Curl, Harold Kroto and Richard Smalley the Nobel peace prize in chemistry in 1996. The hollow nature of buckminsterfullerene makes it ideal for carrying drugs, and releasing them at a targeted cell. Other properties of nanoscale materials also make them ideal

for research into their potential uses in medicine. This branch of nanotechnology often referred to as nanomedicine can be split into 3 main fields of study.

Firstly, techniques in diagnosis and screening could be vastly advanced using nanotechnology, for instance the National Cancer Institute (NCI), have recently announced that researchers have shown that iron oxide nanoparticles have been successfully used in detecting small tumours in the lymph nodes of patients without the use of surgery. The nanoparticles were used with magnetic resonance imaging (MRI) to identify the tumours. (NCI, 2007).

Secondly, the improvement in prostheses and implants. Researchers have found that by using nanofibres on titanium implants as a base coating, it produces a more effective surface for the replacement, allowing the researchers to control the size of the pores in the nanowire. This means that the tissue around the implant can more easily grow and regenerate around the rough surface of the nanowires. This may allow future implants to last far longer than current implants, as these often fail after a period of time due to the smooth titanium on which muscle fibres and other tissues do not adhere easily as shown by the work of Z Ryan Tian (2007).

Finally, the use of nanotechnology in drugs and their delivery. Nanoparticles have been investigated with regards to their tumour targeting capability. This enables drugs to be delivered directly to the site of the tumour therefore only releasing the drug on the site needed. It has been shown that nanoparticles can store drugs very effectively and the release of the drugs can be controlled as shown by Moradi (2005). This kind of drug delivery has been shown to drastically minimise the side effects associated with cancer drugs for instance. The reason for this is that the drugs are usually poorly suited to the internal physiological environment of the human body, however when delivered into the body encapsulated by a nanoparticle, the drug is not in contact with any other body cells so has a minimal effect on cells which have not been specifically targeted.

This largely unexplored area involves the need to penetrate the blood brain barrier (BBB). Currently there is no BBB drug-targeting program in place in global pharmaceutical companies.

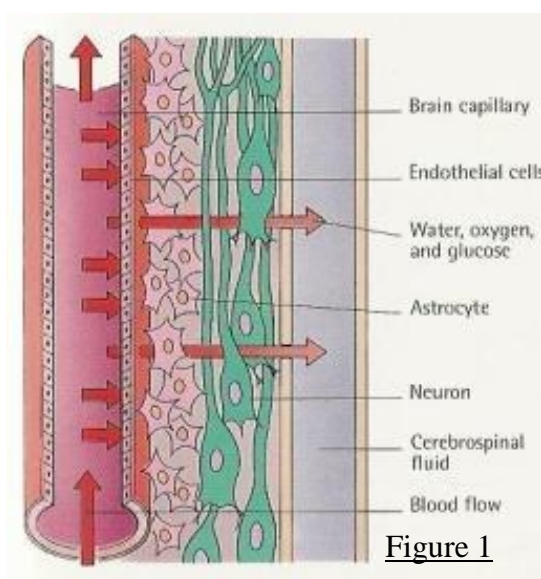


Figure 1

The BBB is shown in figure 1. Its purpose is to separate the circulating blood from the cerebrospinal fluid (CSF), preventing harmful substances entering the brain thus providing a selective anatomical barrier. The BBB is semi permeable and so allows in some molecules and substances but will prevent the penetration of others. All substances and molecules trying to penetrate the BBB must first enter the endothelial cells, as there are no spaces in-between adjacent cells. This provides the cells with greater control over what may penetrate the BBB. Adjacent endothelium cells lining the capillaries in the central nervous system (CNS) form the BBB.

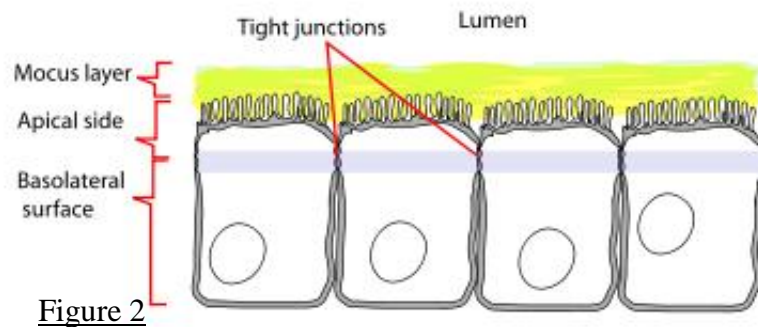


Figure 2

Instead of spaces in-between cells the endothelial cells are joined together by a tight junction.

Tight junctions as shown in figure 2 are located in-between endothelial cells in the

BBB. The tight junctions are held in place by a number of protein complexes that span the length of the endothelial cells. Tight junctions are the points at which endothelial cells are closely associated. These hold the cells together preventing penetration of many substances, and give rise to the high selectivity of the barrier.

Firstly to be able to explore future development I think it essential to understand the properties a molecule needs to have to cross the BBB and the most current research undertaken in the field. Given that >98% of small molecule drugs (approx 400-500 Daltons) and virtually 100% of large molecule drugs (>500 Daltons) cannot penetrate the BBB it is a seemingly large task. The small proportion of drugs that do cross the BBB are highly soluble in lipid and have a low relative formula mass.

Researchers have discovered a possibility to introduce drugs into the CSF crossing the BBB. They have achieved this by temporarily opening the BBB physically to deliver drugs using ultrasound as shown by the work of Perfusion Technology (2011), however this leaves the brain susceptible to infection for a short period of time. The function of the BBB is fully restored after a few hours.

Another method of penetrating the BBB is using the Trojan horse theory. BiOasis technologies have used an iron transport protein to transport molecules over the BBB after having covalently linked the chemotherapeutic agents Paclitaxel and Adriamycin to the protein known as p97 as shown by BiOasis technologies (2008). Although this research is a promising leap into the possibility of crossing the BBB, nanotechnology may lead to other more effective ways of delivering drugs across the BBB whilst being capable of carrying out other functions in the brain.

Discussion

In the next section of this paper I will propose and explore the ways in which nanotechnology could potentially be used in crossing the BBB.

The particles and devices built through nanotechnology would not only have to be effective, efficient and highly specific at their function but would also have to be safe to use in the human body. The devices should have minimal side effects when introduced to the physiological internal environment of the body, but long-term consequences of the intervention would need to be studied in depth. There are also some ethical questions surrounding the possible future developments of nanotechnology. These must be given much consideration in a society that puts great demand on good ethical medical practice, and with its use in neurology being one of

the most controversial aspects, it is important to understand that it may be a few decades before nanotechnology is used routinely in this field of medicine.

Penetrating the BBB is clearly a difficulty in neurological medicine, which needs to be overcome and, when the BBB is fully understood and penetration of drugs is vastly increased, the possibility of treating many diseases of the brain will be dramatically improved. Large molecules need to penetrate the barrier and in order to do this there must be a mechanism in place to carry the particles over the BBB. It may be possible to achieve this by modelling a structure after that of carrier proteins, which are found in cell membranes used for pumping in mineral ions and other substances that cannot diffuse passively. Nanotechnology aims to mimic nature so I think by modelling a theoretical advance in crossing the BBB, after another highly efficient biological mechanism would prove this method feasible.

The first of my ideas and perhaps the most complex is implanting nanodevices in the tight junctions between endothelial cells in the BBB, so that large molecules may be able to be actively transported over the BBB. A nanorobot could be a plausible carrier of larger substances, however at largest a nanorobot would be 100 nanometres whereas the endothelial cells are much longer approximately 10-20 micrometers. Therefore the first step would be to open up the tight junctions using many individual nanotubes that would provide a tunnel like structure. The nanotubes would be ideal because they are exceptionally strong for their size. They are on average approximately 50 X stronger than steel and only 1/10th of the mass. Also the intermolecular forces known as Van Der Waals will affect them. These would help hold the nanotubes tightly together creating the tunnel like structure. These nanotubes can be used as a type of scaffolding in other parts of the body and I anticipate that the nanotubes will behave similarly here although a mechanism to prevent tissue growing around them must be used. I think these may make an ideal material for the mechanism I am proposing, as they are proven to be very conductive as shown by Ballerini and Prato (2008), and so, if required, will be capable of conducting electrical signals through the part of the brain they are penetrating. Also they are capable of establishing close mechanical contact points with cell membranes in close proximity, so any electrical signals in the brain will have minimal interference by the presence of the nanotubes. The nanotubes would provide a channel similar to that of a facilitated diffusion carrier protein but would need to be surgically implanted possibly with the use of keyhole surgery so as to be less invasive. The nanorobot would then be inserted into the hollow tunnel created by the nanotubes and must fit tightly

enough as to not slip. They would need space to rotate when required, in a similar fashion to the mechanism of an active transport protein carrier when it is activated by the breakdown of ATP to ADP and inorganic phosphorus.

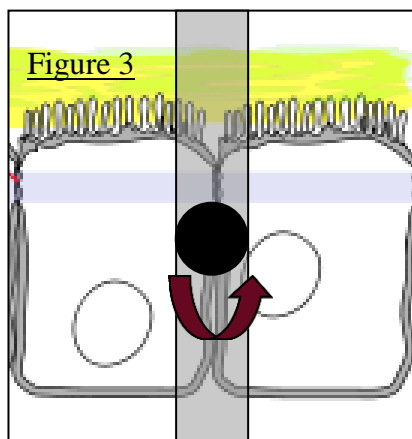


Figure 3 shows a simple diagram of how the nanotubes (shown in grey) would move the cell membranes of the endothelial cells apart and would sit tightly in the gap between the membranes. The nanorobot shown in black would sit between the nanotubes and would move in accordance to the curved arrow.

Some of my ideas to cross the BBB involve nanorobots; in each case the nanorobot will fulfil the criteria discussed in this section, supposing the substance to penetrate the BBB is to be a drug. A nanorobot although not currently used in medical practice today, may have a range of uses in the future and many prototypes have been developed but no working model currently exists. The nanorobots are very much in the research and development phase although some simple molecular machines have been through the first stage of testing. Firstly, nanorobots would need a hollow section so a specified drug could enter it and be transported through the channel. It would also need a store of energy or a simple mechanism to drive the rotation of the nanorobot. It must be stressed that the nanorobot should only let the desired drug through, this is because if other substances were allowed to penetrate the BBB, the brain could be put at risk of infection by chemicals that would otherwise be prevented from penetrating the brain by the BBB. To achieve this I have two possible ideas. Firstly, the nanorobot acts in a similar way to an enzyme whereby it uses the lock and key model. This would entail the nanorobot having a specifically shaped site on it, similar to that of an active site on an enzyme, to which only a specific drug would fit, and so only when there is the presence of the recognized drug would it rotate to transport the drug over the BBB. It may be possible that only one of these structures is needed to effectively transport a sufficient amount of the desired drug over the BBB, but I suspect it is more likely that a number will be needed to achieve the crossing of a drug in any useful concentration. Secondly, a mechanism that would enable the transport of more than one specific drug without the need for more of the same structures with different specifically shaped nanorobots. It may be possible to radiolabel the substances intended to cross the BBB; this would allow many different drugs to pass through the same channel into the brain. It may be possible that the radio labelled drugs are detected by the nanorobot and so it will take only the substances with the radiolabel over the BBB. It may also be possible that a fullerene like nanoparticle could take up the roles that I have proposed.

Secondly, using a similar mechanism to the one above may also be possible, but in a slightly different way, within the endothelial cells instead of between the tight junctions.

Nanorobots may be able to sit in the cell membranes of the endothelial cells, one at either side, acting as two carrier proteins. The nanorobots would act in the same way described above actively transporting identifiable drugs over the BBB. This method does not require the construction of a channel but will transport particles into the endothelial cells and out again at the other end. This method relies upon the fact that because of the tight junctions, no substances can penetrate the BBB without entering the endothelial cells themselves; this is the factor that gives rise to the high selectivity of the BBB. The nanorobots would work in the same way as in the above method but would need to be implanted in a number of the endothelial cells to ensure a large enough concentration of the drug is transported over the BBB. The presence of the nanorobot ensures that the drug would not need to be lipid soluble unlike many other drugs that aim to cross the BBB. Also because of the use of these methods it may be possible that larger molecules would be able to be transported. This is very important as often the drugs and other substances needed to treat diseases of the brain are relatively large.

Finally it may be possible to disguise the nanoparticle and in effect trick the BBB into allowing the particle to pass through it. This would allow a larger concentration of the drug to pass through the BBB to treat diseases such as glioblastoma multiforme (GBM), a disease that is so often hard to treat due to its location behind the BBB. The nanoparticle would take up the desired drug and then be completely coated by a lipid soluble substance that would pass through the BBB with ease when dissociated from the nanoparticle. This may enable the nanoparticle to be transported over the BBB in the same way the lipid would usually be transported, this may be by active transport but more likely passive diffusion depending on the size of the molecule. The movement of the substance over the BBB would depend on the BBB recognizing it as a substance that usually penetrates easily, such as alcohol. The particles would ideally be the equivalent of less than 400-500 Daltons so the coated nanoparticle could penetrate the BBB by passive diffusion across the cell membrane. If the coated nanoparticle equated to more than approximately 500 Daltons it would not diffuse passively through the barrier and another method of penetration would need to be sought, such as active transport or facilitated diffusion. Another factor to consider when choosing the lipid soluble coating should be how smooth the surface will be, as it is less likely to be detected moving through cells. Nanoparticles that could be used in such a method may include nanorobots, quantum rods or a simple molecule similar to buckminsterfullerene all may make ideal carriers.

Although nanotechnology could potentially decrease the size of the drug delivery system it is important to realize that not only the size of a molecule but also the shape could make a significant difference in the penetration of the BBB, and the efficiency within the brain itself. I propose that nanoparticles that aim to penetrate the BBB could possibly be made less of a round compacted shape and could be made to resemble rod like structures. This shape of molecule has been proven to be efficient in the delivery of drugs because it is less likely to be engulfed by macrophages, as they are not wide enough to engulf the particle but also it is less likely to encounter the particle head on as they would move in parallel through the blood stream shown by the work of Joseph DeSimone (2008). These nanoparticles with rod like shapes will be able to carry out the targeting process to correctly identify the cell it aims to destroy or disrupt, but have a greater chance of reaching their destination once in the brain. It is also possible that these nanoparticles may have a shape that is far better at penetrating the BBB unassisted and it may be possible that they could penetrate the tight junctions between endothelial cells and deliver drugs to the brain without any other complex mechanism.

Using this method of crossing the BBB would have minimal impact on the patient, as there is no construction needed to put the system in place unlike the above method. Being that nanoparticles have been shown to be very efficient at taking up and storing drugs, crossing the BBB with a nanoparticle could prove very effective. If a nanorobot was to be used it may also be able to carry out other functions, for example it may be possible it could also take a biopsy from a tumour cell. The nanorobot would also be able to control the amount of drug that it releases at a targeted cell further increasing the concentration of the drug that is directly targeting the correct cells.

All the methods above may not only be used to deliver drugs to the BBB but also used in diagnostic treatments such as delivering substances to fluoresce cells to provide a better contrast on an MRI scan for instance. This may greatly improve the accuracy in gathering data about the size and location in tumours and would be greatly advantageous to surgeons trying to remove such tumours.

Conclusion

In conclusion I believe nanotechnology has the potential to cross the BBB, but I also understand that the BBB must be further understood and nanotechnology must further advance before an effective drug delivery system is seen to effectively and efficiently penetrate the BBB. There are obvious limitations in my work and as I have only looked theoretically at the possibility of crossing the BBB there is likely to be flaws.

The devices obtained through nanotechnology must be safe to use within the human body, have minimal side effects to the patient, and the benefits of such a device must out way the risks by some margin. The safety of the patients must always be the first priority of any doctor and I have found a possible problem in my work concerning this. Devices formed through nanotechnology must carry out a specific function completely independent from the functions that will be occurring in surrounding tissues thus not interfering with any other biological process. With the uses of nanotechnology in its infancy the long-term effects the materials can have on the human body is widely unknown. For example straight carbon nanotubes of approximately 20 micrometers in length have been shown to resemble asbestos fibres and could potentially cause the formation of some cancers as shown by Maynard (2009). Although there is some strong scientific evidence for this theory far more information will be needed to determine definite side effects of the carbon nanotubes.

Undoubtedly nanomedicine aims to improve the diagnosis, treatment and prognosis of patients, but with the brain being one of the most delicate and important organs in the body, an error in the use of nanotechnology could be fatal. Clearly methods of treatment would not go ahead without extensive clinical trials, however how are we to know the long term affects and is it right to use such techniques with the limited information we have on the materials? On the other hand the advance in nanomedicine will ultimately rely on the fact that it has to be tested at some point and in my opinion the prospects of nanotechnology in medicine is so great that it would be foolish to slow their development.

References

Books

Tortora, G. J., Grabowski, S.R (1996) Principles of Anatomy and Physiology, Harper Collins College Publishers (397)

Wilson, K.J.W., Waugh, A (1996) Ross and Wilson Anatomy and Physiology in Health and Illness, Churchill Livingstone (146-149)

Web Addresses

Blood Brain Barrier www.daviddarling.info/encyclopedia/B/blood-brain_barrier.html

Blood Brain Barrier Drug Targeting: The Future of Brain Drug Development
molinterv.aspetjournals.org/content/3/2/90.full

Carbon Nanotubes www.personal.reading.ac.uk/~scscharip/tubes.htm

Crossing the Blood Brain Barrier with Nanotechnology
www.nanowerk.com/spotlight/spotid=6269.php

Current Status of Nanomedicine and Medical Nanorobotics
www.nanomedicine.com/Papers/NMRevMar05.pdf

Engineered Magnetic Nanoparticles Image Small Tumours
nano.cancer.gov/action/news/2007/jan/nanotech_news_2007-01-22b.asp

Ethical Aspects of Nanomedicine www.capurro.de/nanoethics.html

For Nanotech Drug Delivery Size Doesn't Matter—Shape Does
www.scientificamerican.com/article.cfm?id=size-shape-matter-nanotech-drug

Glioblastoma www.irsa.org/glioblastoma.html

Lipid-Coated Nanoparticles Yield Breathable Anticancer Agent
nano.cancer.gov/action/news/nanotech_news_2006-05-15c

Nanomaterials: It's a Small Small World
www.csa.com/discoveryguides/nano/overview.php

Nanomedicine-Overview commonfund.nih.gov/nanomedicine/overview.aspx

Nanoparticles Cross Blood Brain Barrier www.scientistlive.com/European-Science-News/Nanotechnology/Nanoparticles_cross_blood-brain_barrier/23115/

Nanorobots ewh.ieee.org/r10/bombay/news3/page4.html

Nanotechnology Approaches to Crossing the Blood Brain Barrier and Drug Delivery to the CNS www.biomedcentral.com/1471-2202/9/S3/S4

Nanoparticles Cross Blood Brain Barrier www.scientistlive.com/European-Science-News/Nanotechnology/Nanoparticles_cross_blood-brain_barrier/23115/

Nanotechnology Leads to Better Bone Implants
www.zdnet.com/blog/emergingtech/nanotechnology-leads-to-better-bone-implants/677

Nanotechnology: The Smaller the Better
serendip.brynmawr.edu/biology/b103/f00/web1/plotnick.html

Nanotechnology to Repair the Brain www.nanowerk.com/spotlight/spotid=8760.php

Nanotechnology to Revolutionize Drug Delivery www.in-pharmatechnologist.com/Materials-Formulation/Nanotechnology-to-revolutionise-drug-delivery

New Carbon Nanotube Study Raises the Health Impact Stake
<http://2020science.org/2009/03/26/new-carbon-nanotube-study-raises-the-health-impact-stakes/>

Opening up the Brain with Ultrasound
www.technologyreview.com/biomedicine/37451/?a=f

Some Nanotubes Could Cause Cancer www.technologyreview.com/Nanotech/20815/

‘Trojan Horse’ Crosses Blood Brain Barrier
www.in-pharmatechnologist.com/Materials-Formulation/Trojan-horse-crosses-blood-brain-barrier

What are Nanorobots www.wisegeek.com/what-are-nanorobots.htm

25 Ways Nanotechnology is Revolutionizing Medicine
mritechnicianschools.net/2010/25-ways-nanotechnology-is-revolutionalizing-medicine