

The Use of Nanotechnology
For the Regeneration
Of the Central Nervous System

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PASS WITH MERIT

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Abstract

In this paper, we shall explore the possibilities behind the applications of Nanotechnology in medicine; specifically centred upon damage to the spinal cord. Whilst the current levels of technology and treatment make full or even partial recovery from such injuries very unlikely, we believe that with further developments in Nanotechnology and the synthesis of organic molecules new methods for dealing with such devastating injuries can be developed.

Our project will explore the possibilities behind creating nanofibre scaffolds, self assembling protein structures and implanting them into the patient's body and the possible effects that this could have on trauma to the spinal cord at a cellular level. We conclude that the technology we discuss is certainly feasible and that the potential effects of these technologies on regenerative medicine are huge but need to be researched and understood more fully than they are at present.

Introduction

Nanotechnology is the idea of engineering and constructing systems at a molecular level; "Building things from the bottom up" ¹. Increasingly sophisticated advances in chemistry and physics are generating exciting new nanotechnologies.⁵

The word "nano" originates from the Greek word meaning "dwarf". 1 nanometre is 1 billionth (10^{-9}) of a metre or equivalent to the length of 10 hydrogen atoms. Working at such a small scale has led to the development of a variety of new materials with highly controlled and interesting properties including exceptionally high strength polymers and even given some nano-based materials the ability to behave in a similar way to the body's native proteins.⁵

A large number of diseases and medical conditions exist at a cellular level. Using nanotechnology to help tackle the very root of some of these problems could be highly effective through allowing us to manipulate systems at a molecular level. Nanoparticles are similar in size scale to many common biomolecules⁵. This therefore makes it possible for nanoparticles to be used for the delivery of drugs, heat, light or other substances to specific types of cells (such as cancer cells)³. There is the possibility of using nanobots for repairing diseased cells. These nanobots are able to manipulate molecules and atoms⁴ and act in a similar way to the body's natural antibodies.

Currently, nanotechnology is very much an evolving science and many of its applications in medicine are purely theoretical or possible in laboratories. These applications, once made widely available, could vastly improve the treatment for conditions that the medical profession currently has very limited answers too.

With our current level of technology, cancer is very hard to treat safely and effectively. Treatments such as Radiotherapy and Chemotherapy all hold large risks and levels of discomfort for the patient yet are the only existing methods for the treatment of this all too common disease. Nanotechnologies in development include 'smart drugs', or nanostructures designed to target only specific cells within the body and deliver either drugs or another resource to them. These 'smart drugs' are designed so that the substance to be delivered is encased in a biodegradable shell such as a Buckyball (Buckminsterfullerene). These are Carbon compounds that are hugely structurally different to diamond or coal or any other naturally occurring C compound. Built from 60 Carbon atoms, Buckyballs are spherical, hollow and highly resistant. These properties make them ideal to carry drugs around the body for release in certain areas.

One particular area of interest to us is the use of nanostructured scaffolding for tissue engineering in regenerative medicine¹³. This particular area of research could potentially aid the regeneration of the central nervous system, solving the problem of paralysis due to spinal injuries.

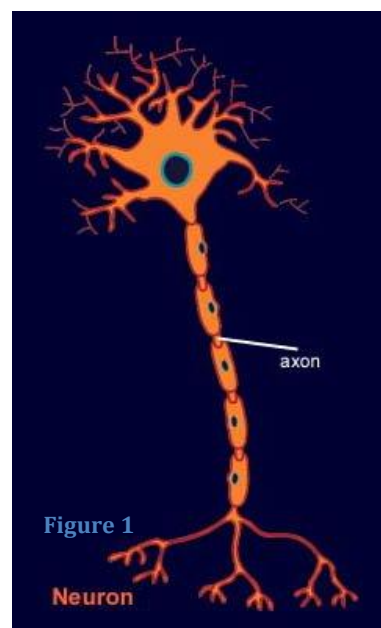
The specific application for nanotechnology in medicine that is the basis of our paper is using Nano-fibrous scaffolding to help the Central Nervous System (CNS) regenerate. Under natural conditions the CNS has almost no capacity to re-grow after damage, particularly heavy trauma (normally resulting in paralysis). The Peripheral Nervous System (or PNS) on the other hand has a fairly large ability to re-grow itself with axon growth appearing very quickly after the initial trauma. Whilst the reason to these differing behaviours is not fully understood, it is believed that the conditions in the spinal cord and cranium are not conducive to nerve growth, and that 'Glial scarring' occurs after damage. This scarring prevents the axons growing, resulting in permanent damage of the spinal cord. It is our belief that by using nano-scaffolding and some spinal surgery it would be possible for the spinal cord to re-grow after injury and that this capability would have many applications in CNS disorders such as paralysis and Multiple Sclerosis.

Discussion

The central nervous system comprises of the spinal cord and the brain, two of the most vital organs in the body. The central nervous system coordinates activities between various parts of the human body. It works in collaboration with the peripheral nervous system and plays a fundamental role in controlling the behavior in multicellular organisms.⁷ The brain is protected by the skull and the spinal cord protected by the vertebrae, however these organs are still susceptible to injury, and it is common for the spinal cord to be damaged by the spinal column itself. Such injuries can commonly cause complete paralysis.

When the central nervous system is damaged it cannot regenerate unlike the peripheral nervous system. After an injury to the spinal cord has occurred, immune system cells accumulate in the region of the injury, these immune system cells invade the lesion causing cavitation to occur at the centre of the lesion. Due to this cavitation, the alignment of nerve axons in the spinal cord is disrupted. After this disruption these axons (seen in figure 1) attempt to re-connect themselves by undergoing mitosis and hypertrophy. However, the nerves attempts of regeneration results only in the formation of Glial scars. This thick fibrous tissue stops the axons from regenerating; this is because when they encounter the Glial scar, dystrophic end-bulbs form.

As a result of the immune response to the trauma, substances are released from the cells which cause the death of Oligodendrocytes resulting in the Axons losing their Myelin sheaths. Myelin is a protein-lipid complex made up of many layers of the cell membrane of Schwann cells⁸. The myelin sheath is a protective cover that surrounds nerves and



neurons in the brain and spinal cord acting as an insulator for the axons and increases the speed at which electrical impulses are transmitted along the axons. Damage to the sheath can slow or stop nerve impulses. This can result in partial or complete loss of sensation and function in certain areas.

There are also several other diseases which can damage the central nervous system. An example would be Multiple Sclerosis (MS) which is a common auto-immune, demyelinating disease. The body's immune system attacks the myelin sheath or cells that produce and maintain the sheath. This can cause inflammation and cause injuries to the nerves that the sheath surrounds. The formation of Glial scarring occurs in the same way that it does following spinal injuries, hence preventing the regeneration of nerves.

There have been many areas of research involving the regeneration of nerves after injuries to the central nervous system. Existing methods include the use of stem cells, the grafting of peripheral nerve pieces and fetal tissue to re-connect damaged areas in the spinal cord, and several other methods. In this paper the use of nanofibres to form scaffolding for nerve axons will be discussed.

How does the Nanoscaffolding work?

Nanoscaffolding is a sub branch of nanotechnology and focuses around building 'scaffolds' from nanofibres. These scaffolds could be used in many different commercial areas including Composite Materials, Environmental Sciences and the Life Sciences including medicine. By using different materials to build the nanofibres themselves, properties relevant to the proposed application for the scaffolding can be fabricated. Examples of this include a highly porous material for use in performance clothing for athletes or easy control over the size of the spaces between fibres that could be used for separating solutions, or even to provide safe and clean drinking water.

In order to understand the properties and construction of Nanoscaffolding and how we believe it could be applied to the problem of regeneration in the CNS we must first understand the basic components that are used to fabricate it, these components being the nanofibres.

Any fibre with a diameter of less than 1000 nanometres is classified as a nanofibre. At the current time the majority of nanofibres produced are made from inorganic substances such as Titanium Dioxide and Silicon Dioxide, ionic materials known for their large strengths¹⁴. The technology to produce such fibres currently exists and several companies are marketing nanofibre products for the reasons stated above. These inorganic fibres are created by the process of electro spinning; involving the electrical charging of the surface of a liquid until very thin streams are expelled and dry in the air, forming regular, uniform fibres¹⁴. Nanofibres created in this way display huge tensile strength in comparison to their weight; outperforming steel many times over. The scaffold they form is porous, with gaps between the strands and this would allow the axons from damaged nerve cells to use the scaffolding as a template to extend across. The innate ability of cells to regenerate using Nanoscaffolding has been demonstrated in many other, if less complex areas of the body. However, whilst these ceramic based scaffolds work for regular tissue, axon re-growth is somewhat more difficult to achieve. Also these materials are inorganic, and whilst they may be broken down by the body in time the body cannot make use of the compounds.

The very latest in Nanoscaffolding technology is being studied at Harvard University. Self assembling peptides have been created in laboratory conditions and these molecules, formed from peptides (short chains of amino acids) are totally organic and

once created will assemble themselves into scaffolding without the need for human intervention¹¹. This means that if they were inserted into the damaged area of the spinal cord the incision could then be closed and the polypeptide would assemble itself into the required scaffold to allow for axons to begin extending and knitting together. The Nanoscaffold would therefore be made entirely from organic components and should cause no irritation or adverse effects to the patient's body. Once the process of re-growth is complete the body should break down the polypeptides leaving only amino acids behind which the cells around the area can then use in a beneficial manner.

The application of Nanoscaffolding in the central nervous system

The Nanoscaffolding used is called "Self-assembling peptide nanofibre scaffold (SAPNS)"⁹. We believe that the SAPNS could be made to be self assembling in the same way that proteins assemble in the body. After the amino acids have been correctly ordered and the primary structure created, the SAPNS could be implanted in the body (whether directly or through a vector will be discussed later in this paper). After implantation the specific structure of the SAPNS, especially the "R" variable groups will cause it to self assemble into the required scaffolding structure. This self assembly occurs in the same way a naturally synthesised protein would assume its

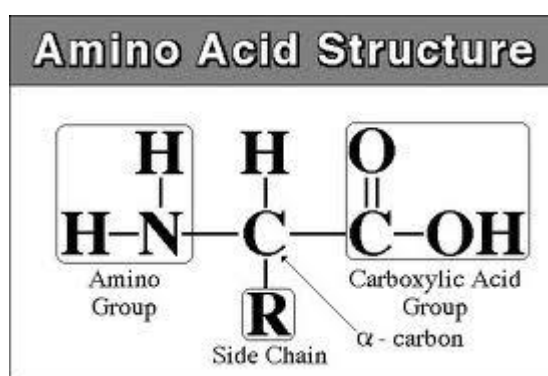


Figure 2

required shape. The Secondary Structure is caused by hydrogen bonding between the amino acids; resulting in a helix or pleated sheet structure. For the purpose of Nanoscaffolding we suggest that a pleated sheet structure is implemented due to the possibilities to form regular, porous scaffolding suitable for axons to re-grow over. After the Secondary Structure is formed the variable "R" groups attached to each amino acid interact, forming many new bonds and shaping the SAPNS into its Tertiary Structure. Figure 2 shows the structure of an amino acid. The order of amino acids in a polypeptide chain will determine the shape of the protein. This is because the variable "R" group found in the amino acid can be attracted to the "R" group of amino acids and can cause the polypeptide chain to fold to form a certain shape. Disulphide, Hydrophilic/phobic and Hydrogen bonding are all present at this stage. The "R" groups that result in a suitable matrix for Axonal re-growth would have to be carefully chosen and researched lest a less desirable shape is achieved.

Because these fibres are nanoscale, there is likely a direct interaction between the peptide scaffold, the extracellular matrix, and the neural tissue on both sides of the lesion. These nanoscale fibres create a scaffold that connects the two faces of the lesion, allowing the movement of cells into the scaffold. The peptide scaffolds create a permissive environment for axonal growth while discouraging or preventing the scar formation that normally occurs at an early stage.⁹

We have discussed two methods for the introduction of the protein scaffolding into the patient's body and both have their merits;

Implantation through surgery could prove highly effective in the spinal cord although would require advanced methods to operate on such a small scale. If the surgeon were able to operate on such levels as to implant the protein then it should also be possible for the surgeon to physically remove any scarring from the cavity formed after the injury occurred. Once the scarring has been removed, the protein can be implanted in its

place. The protein would assemble itself into a scaffold to bridge the disrupted area in the spinal cord and promote axonal growth.

Unfortunately such precise surgical methods do not currently exist or at least are not widely available and we were therefore pressed to think of another method for the introduction of the SAPNS. Were the SAPNS suspended in a solution (possibly saline or de-ionised water, as these are often used in surgical procedures and are benign to the body) then it could be injected into the cavity, filling it¹³. The drawback to this method is that, as far as we could derive from our knowledge it would do nothing to prevent Glial scarring if the Axons did not begin to immediately extend. Given the current level of surgical technology, we believe that this would be the most viable method for the successful implantation of the SAPNS into the patient's body.

For successful axon re-growth, the axons must be able to find appropriate targets¹⁰. The nanofibres would hopefully provide a bridge to make connections between the nerve cells and act as a guide. The growing axon and its target must interact to construct a synapse, a specialized structure that acts as a connection between nerve cells. After the Axons have fully extended and hopefully knitted together, the body will no longer require the scaffolding to remain in place. Unlike a ceramic or metal based Nanoscaffold which is non organic and would take a long time to break down, the SAPNS is constructed in the same manner and of the same matter as any other protein in the body. Thus the body can break it down in the same way, breaking it into individual amino acids which can then be further used by surrounding cells. The entire process from implantation to degradation should be totally neutral, if not slightly beneficial to the body as a whole. All the molecules used are biological and would occur naturally in the body anyway making any complications or poisoning very unlikely. Due to the scale of operation needed, no matter which of our two introductory methods was implemented, the risk of infection is smaller than many operations as any incision need only be very small.

One of the reasons that we chose to investigate and discuss the possible applications for spinal regenerative nanotechnology was the large number of incredibly difficult to treat and life changing afflictions that are caused by damage to the central nervous system.

Whilst the majority of our reasoning and research in this paper has been directed towards the regeneration of the spinal cord following severe trauma and the subsequent paralysis of the patient, there are many other conditions that this technology, were it made a reality, could help to effectively and safely treat. The SAPNS could just as easily be introduced into the brain of the patient to allow brain tissue to re-grow in a damaged area as this tissue behaves in almost the exact same way as that found in the spinal cord¹³. This might allow patients with degenerative cognitive conditions such as Alzheimer's Syndrome to potentially recover at least partially.

With some minor modifications we believe that the SAPNS could help patients with Multiple Sclerosis, a condition in which the Myelin sheaths of Axons in the brain and spinal cord are attacked by the body's own immune system. Were the SAPNS capable of helping not just full axons re-grow, but the Myelin Sheaths individually then this treatment could prove highly useful in this field as well.

Ethical Issues surrounding the use of nanotechnology

Societal risks from the use of nanotechnology have also been raised. On the instrumental level, these include the possibility of military applications of nanotechnology as well as

enhanced surveillance capabilities through Nano-sensors. This application could also be modified to a civilian level. Implants that monitor chemical levels or such in the body transmit the data back to a central database, in theory taking away confidentiality and some human rights from the patient, this leads on to the question of whether our health system could handle such huge quantities of data effectively and whether these files could be completely safe.

Concerns have also been raised that the benefits of nanotechnology will not be evenly distributed and that any benefit associated will only reach the countries where it has been developed, these countries which are mainly USA, Japan, France and Germany which all have huge economy's and complex health care systems in place and have both the facilities and the funding to invest heavily into such technological advances. In addition it is unlikely that developing countries will have access to the funding, infrastructure and human resources required to support the development and research of nanotechnology.

There is obviously also the possibility of miniature weapons and explosives being manufactured and the capabilities of current weapons being increased. Also there is the possibility of creating biological weapons that affect organisms at a molecular level. Such weapons could be catastrophic and could result in major disasters.

Both the advantages and disadvantages of nanotechnology must be considered. It is evident that the use of nanotechnology can bring major benefits to society in many fields, including medicine. It is important that research in nanotechnology continues for the development of useful purposes. However it would be important for there to be guidelines to be set to ensure that the use of nanotechnology is not used for inhumane purposes such as the manufacturing of biological weapons.

Conclusion

In conclusion, the potential and possibilities for the use of nanotechnology in medicine is vast. This paper has highlighted a specific area of the development; nanoscaffolding for the regeneration of the central nervous system. The peptide scaffolds provide a bridge for the re-growth of axons. We believe that the use of these Nanostructures can increase the quality of life for paralysed patients, by allowing these patients to possibly regain sensation and function to certain areas, after paralysis which may be due to blunt trauma, multiple sclerosis or other diseases affecting the central nervous system.

However, as mentioned earlier a potential problem has occurred in the delivery system of this new medicine. The use of surgery is an obvious choice, but the implication could be large as any type of surgery has its apparent risks and dangers to the human body. Not to mention the fact that this is such a specialized field of medicine that numerous hospitals and health centers don't have the facilities to incorporate the use of such tiny substances in surgery. Meaning health care of this kind is limited to a chosen few. The second kind of delivery system mentioned is the use of injection into the affected area through the use of solution possibly saline or de-ionised water, as these are often used in surgical procedures and are benign to the body. Unfortunately this model of implantation does not solve the problem of Glial scars forming. However through time we hope these problems can be overcome to allow patients in all areas to receive this type of medical treatment at their convenience.

If it became possible for surgical procedures to be performed at a nanoscale, there is the possibility of being able to deliver the SAPNS to exactly where it is needed. This would have two advantages, the first of which would be it is easier to administer the SAPNS and make sure that it constructs itself in a manner that would aid the regeneration of axons. The second advantage would be a decrease in recovery time both saving hospital money and the risk of a post-surgical infection.

We can be sure that there will be no negative long term effects to the body that are due to the use of the nanofibres. Because the molecules used in the nanostructure are all organic and used in the human body extensively, there is no risk of poisoning or long term damage as these nanofibre structures will degrade over time and the components could possibly be used by surrounding cells.

We can be optimistic for the future of this specific area of medicine as we have shown there is the scientific basis for these techniques to be used. There are some obstacles which must first be overcome and still lots of research to be undertaken. Once these obstacles have been overcome we can expect to see this particular method being used to regenerate damaged nerves following an injury to the central nervous system.

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