Nanotechnology in medicine:

The application of nanotechnology in the regeneration of nervous tissue

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Abstract

This paper presents some of the uses and benefits of nanotechnology in regenerative medicine. The main focus is the discussion of possible benefits of applying nanotechnology in the process of regeneration of nervous tissue damaged in strokes and Central Nervous System (CNS) injuries. Based on current research, it explores how recent research findings can be used in the treatment of nervous tissue damage and possible areas of future developments. It discusses the ethical and social issues that will arise as a result of progress in this new field of applied science. Nanotechnology opens a possibility for us to interfere with substances on a molecular level and this could have immense impact on our daily life.

Introduction

Nanotechnology can be defined as "engineering of functional systems at the molecular level". It refers to development and application of materials, devices and systems delivering new properties and functions because of their structures in the range of about 1 to 100 nanometres. [20]

Richard Feynman, a Nobel Prize winner in Physics, envisioned the capability of nanotechnology in 1959: "*The principles of physics, as far as I can see, do not speak against the possibility of manoeuvring things atom by atom. It is not an attempt to violate any laws; it is something, in principle, that can be done; but in practice, it has not been done because we are too big."*

Some experts may still insist that nanotechnology can refer to measurement or visualisation at the scale of 1-100 nanometres, but it seems that the ability to control and organise the material at nanoscale is an essential element of nanotechnology.

Due to the fact that it reaches the level of atoms and molecules, nanotechnology provides unifying concepts for research which can be used broadly. Nanotechnology can be applied to many areas of research from medicine, through textile, food industry, cosmetics and environment to computer industry. What is interesting is that many applications in nanotechnology have been created by scientists carefully examining "a living world" to find better solutions for "a non living world". Due its universal concepts, this field shows rapid progress.

Mihail Roco of the U.S. National Nanotechnology Initiative has introduced the concept of "four generations" of nanotechnology development (Figure 1: Roco 2004 - see chart below).

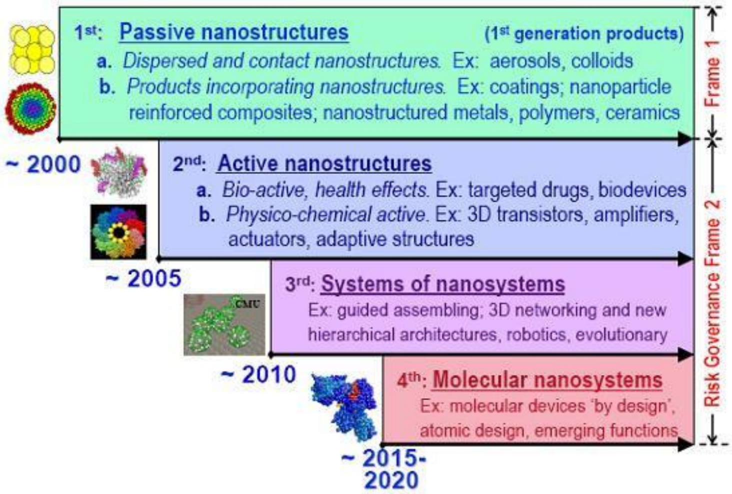

Figure 1: The four generations of nanotechnology development

Examples of nanostructures, described as the first generation of nanotechnology, include joint replacement using biocompatible nanostructured materials and diagnostic methods using quantum dots and nanoparticles. Examples of second generation nanotechnology products include localised drug delivery, as a way to deliver medicine to target cells, making the treatment more effective and limiting side effects.

Regenerative medicine is one of the potential fields where nanotechnology applications can be widely used. It is defined, by the Centre for Regenerative Medicine, University of Bath, as "an emerging interdisciplinary field of research and clinical applications [which] focuses on the repair, replacement, or regeneration of cells, tissues, or organs." Neuroregeneration refers to the restoration of nervous cells and nervous tissue. Nervous cells and tissue can be damaged through different mechanisms from spinal cord injuries and strokes to neurodegenerative conditions.

A spinal cord injury is an "insult to the spinal cord resulting in a change, either temporary or permanent, in its normal motor, sensory, or autonomic function" (Segun T Dawodu 2008). The injury causes a compression of the vertebrae, intervertebral discs (as shown in figure 2) and damage to the axons, which are responsible for carrying signals from the brain to the rest of the body.

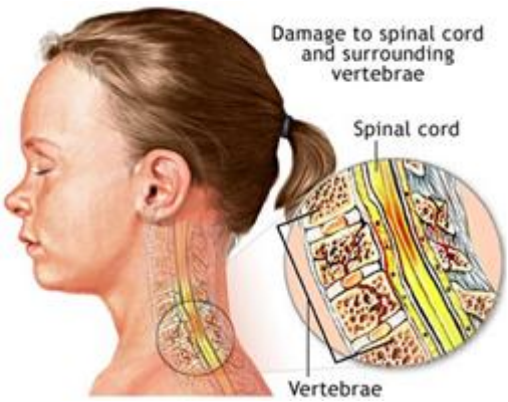

Figure 2: Mechanism of spinal cord injury

The extent of spinal injury is variable, depending on the level of injury and the extent of damage caused to axons. Spinal cord injuries are commonly caused by trauma from every day accidents, like car crashes as well as sport and war injuries. Some injuries have a complete recovery; unfortunately others will lead to severe permanent functional impairment like quadriplegia. In the United Kingdom there are currently 40,000 paralysed people, and thousands more who suffer from the effects of spinal cord injuries. There are huge financial implications of providing medical care for individuals with spinal injuries.

Stroke is defined by the World Health Organization as a clinical syndrome consisting of 'rapidly developing clinical signs of focal (at times global) disturbance of cerebral function, lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin'. Common mechanisms are: occlusion of cortical arteries, embolism and arterial thrombosis.

The extent of damage to neurons depends on the severity of ischemia. If supply of oxygen to the neurons is insufficient but they are not permanently damaged, quick restoration of blood flow may reduce or reverse ischemic injury.

Stroke is one of the biggest health problems in the UK. Annually, 110,000 people suffer from first or recurrent stroke and over 56,000 die as a result. [12] Over 900,000 people have to live with effects of stroke and half of them require help with daily life. [13]

Strokes have a huge implication on England's economy, costing around £7 billion per annum, including treatment, care and cost of lost productivity. [12]

Discussion

Victims of stroke and traumatic brain and spinal cord injuries can recover from their injuries through rehabilitation although they are often left with permanent disabilities. CNS has a very limited ability to regenerate, following an injury. The repair of CNS is strongly inhibited by glia forming scars and producing factors inhibiting axon repair and remyelination. The axons have the capacity to grow again but are unable to do so because of scar tissue. Injuries to the spinal cord result in disruption to the neuronal membranes, followed by extensive secondary neurodegenerative processes. The Northwestern University researchers showed that a nano-engineered gel can reduce the development of scar tissue at the site of spinal cord injury. The gel was tested on mice with a spinal cord injury and after 6 weeks there was a significant improvement in their motor function. The gel was injected into the spinal cord and assembled into a scaffold, allowing nerve fibres to regenerate. Research on animals shows that polyethylene glycol, administered within 72 hours, can be an effective way of treating spinal cord injuries. [11] Polyethylene glycol is a sealing agent that, when injected, seals the holes in the damaged nerve cells. This allows healing of the cells to take place. Nanotechnology provides a more effective approach to this style of treatment of spinal cord injuries. Injured axonal membranes can be repaired using di-block copolymer micelles. Tests conducted on rats show that spinal tissue incubated with micelles (60nm diameter) demonstrated rapid restoration of compound action potential and reduced calcium influx into axons. These were much lower than the concentrations of polyethylene glycol. Intravenously injected micelles resulted in recovered locomotive function. As a whole, copolymer micelles can minimise the damage, following a spinal cord injury, with minimal toxicity. [10][19]

Nanotechnology can be used in damage of brain tissue. Experiments have been conducted at MIT, Hong Kong University, and Fourth Military Medical University in China, where researchers first cut into the area of the brain that conveys signals for vision, causing the small laboratory animals to be blinded in one eye. Chains of amino acids were injected into the brain, causing the peptides to bind to each other, creating nanoscale fibres that were able to bridge gaps caused by the damage. It is also thought that the nanoscale mesh, created by the fibres, may encourage cell growth and prevent scar tissue from forming. As a consequence of the nanoscale bridges, symptoms previously shown from damage to neurons in the brain that may come as a result of a stroke or traumatic brain injury were prevented. In the case of the experiment the researchers performed, 75% of the animals were able to see well enough to detect and turn towards food. [2]

Stem cell transplant has been recently used in neuroregeneration. Stem cells are able to divide by mitosis and then differentiate into many different cell types. They allow healthy neurons to be made in brain-damaged patients so re-establishing brain activity. The main problem when using stem cells, however, is that they tend to travel to healthy areas of the brain instead of the areas where they are needed. This is because healthy neurons produce proteins that attract stem-cells away from “unhealthy” areas. The use of carbon nanotubes is thought to help with this matter. Carbon nanotubes are allotropes of carbon, with a cylindrical nanostructure. They act as scaffolding by holding the stem cells in the ‘unhealthy’ regions whilst also appearing to play an active role in turning stem-cells into neurons. [3] Neurones and carbon nanotubes have similar properties. They both have similar shapes; carbon nanotubes are a cylindrical shape and neurons are in elongations. Since carbon nanotubes are also conductive, in theory they could be used as a neural implant material. Their conductivity would allow electrical signals to be transmitted to the neurons. As well as acting as ‘scaffolding’, carbon nanotubes can also be used to grow nerve cells. Researchers in Italy have developed a way of growing nerve cells from the hippocampus region of the brain on substrates containing networks of carbon nanotubes. Tests have been conducted in which neurones have been grown on coated and uncoated cover slips. They both produced similar growth, electrophysiological characteristics and similar intrinsic excitability. However, only the one, grown on the carbon nanotube coated slip, showed a significant increase in the frequency of postsynaptic currents. This development identifies that using carbon nanotubes results in a large improvement in the efficiency of signal transfer in neurones and, with further development; electrical signal transfer can significantly improved in injured patients. [9]

When treating CNS disorders, the main problem is usually getting drugs across the blood-brain barrier. The blood-brain barrier separates the circulating blood and the brain’s extra cellular fluid, helping to maintain homeostasis by preventing the entrance of harmful substances and allowing the entrance of essential nutrients. The barrier is a tight seal of endothelium cells that line the blood vessels in the brain so allowing it to be selectively permeable. The blood-brain barrier only allows non-ionic and low molecular weight substances that are soluble in lipids to cross because of the physical and metabolic barriers present. [6]

The difficulty in crossing the blood-brain barrier provides a major problem when trying to get drugs across for stroke treatment. Recently, however, a new treatment has been developed by X-Cell Centre where stem cells are injected into the blood stream along with Mannitol. Its diuretic properties help by removing fluid from the endothelium cells causing them to shrink thus creating greater gaps between the cells. Opening the blood-brain barrier, however, is risky because making it less effective could let in not only stem cells but many other molecules in the blood that are harmful to the nervous system. Therefore, using nanotechnology could prove a more effective way of crossing the blood-brain barrier. [24]

A nanoparticle-based platform, which uses the 'trojan horse' concept, offers the possibility of crossing the barrier without damaging the blood-brain barrier as a whole. The Trojan horse concept works by attaching whatever is needed to cross the barrier to a substance that is able to use a receptor in the blood-brain barrier. (Figure 3)

Once the substance binds to the receptor it is then able to carry the substance across along with an attached substance. For example, certain proteins and peptides, like the iron-transporting protein Transferrin, are able to cross the blood-brain barrier. Their job is to work as carriers of essential nutrients into the brain. Linking Transferrin with nanocrystals (quantum rods), allows the quantum rods to get across the blood-brain barrier into the brain. This technique provides the potential to allow diagnostic and therapeutic agents to enter areas where they are required. A scientist, Indrajit Roy, University of Buffalo developed the system: *"Our findings unfold a new dimension in blood-brain barrier transport using inorganic nanoparticles, which are structurally robust and demonstrate the potential to transport multiple agents across this physiological barrier"*. Quantum rods that are used in this way have low toxicity so, when linked with drugs they act as probes targeting diseased cells of the brain. In theory, this method could possibly be used for the transportation of stem cells and other regenerative drugs, thus providing stem cell treatment to the CNS without compromising the brain's natural defence, the blood brain-barrier. [1][23]

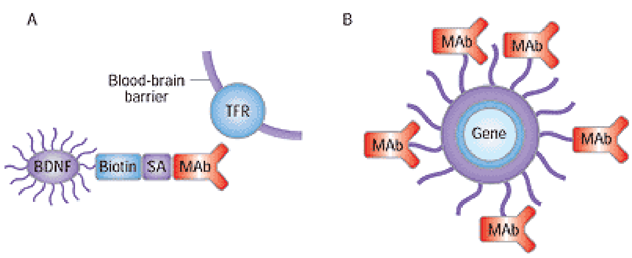

Figure 3: Trojan horse designs. Two different delivery architectures used for brain drug targeting. (A) Brain-derived neurotrophic factor (BDNF) is linked to a monoclonal antibody (MAb), which binds to the transferrin receptor (TFR) on the BBB, via streptavidin (SA)–biotin chemistry. The BDNF is surrounded by strands of polyethylene glycol (PEGylated), which inhibit the uptake of the drug by peripheral organs, particularly the liver. (B) Double-stranded plasmid DNA is encased in a liposome that is attached to PEGs that bind the MAb transport vector for nonviral brain gene delivery. (Adapted from *Nat. Rev.: Drug Discovery* **2002,** *1,* 131–139.)

Nanotechnology can also be used to provide more effective microstimulation. This is a technique that stimulates a small population of neurons by passing a small electrical current through a nearby microelectrode. It is thought that through use of this technique, a method can be developed to deliver sensory percepts to damaged sensory receptors or pathways. An example of this is when a part of the brain called the primary visual cortex is stimulated to create flashes of light in blind people. Through this method of treatment it can help to restore some vision for blind individuals. This idea, in theory, could lead to an effective treatment for spinal cord injuries. Nanotechnology offers the possibility to develop smaller electrodes to provide spinal microstimulation without causing as greater damage to neurones.

Neuroprotection is another area in which nanotechnology can be used for neuroregeneration. It is a strategy used to protect against neuronal injury or degeneration in CNS following acute disorders. Substances such as superoxide (O2-), hydroxyl (OH), peroxynitrite (ONOO) and peroxide (H202) lead to degenerative changes in cells, including DNA fragmentation and peroxidation of cell membrane lipids. To deal with this, carbon 60 fullerene based neuroprotective compounds are being developed. Fullerene is a molecule composed entirely of carbon. These molecules are composed of large three-dimensional arrays of evenly spaced carbon atoms. Fullerenols are the alcohol equivalent to fullerene, with a hydroxyl group. They possess antioxidant and free radical properties. They have an ability to reduce cell death and appear to lower intracellular calcium concentrations, a vital mechanism of excitotoxicity in neurons. [21]

As presented above, there are several ways of applying nanotechnology in treatment of stroke and CNS injuries, as a way to overcome current limitations of treatment. It could prove to be an effective treatment but requires further testing to confirm its efficiency and safety.

Ethical and social issues

There are many ethical issues around nanotechnology that have to be explored and tackled in order to continue with development of this field of applied science. Firstly, many religious people would oppose this technology on religious grounds. It can be seen as 'playing God', as nanotechnology involves the introduction of minute, unnatural devices into the human body. This could indicate that the human body is imperfect and that could suggest that the creator, God or Allah, is also flawed. Another potential issue associated with nanotechnology is the fact that we do not know the long-term effects of this new technology on animals and definitely not on humans, there is concern that the new developments of treatments could be more harmful than those already in use. Linking to the main topic of this paper, an experiment on a hamster showed that nanotechnology can be used in restoring gaps in the nervous system and any minor tissue damage to the brain, since the treatment restored the hamster's eyesight. Even though that particular experiment was successful, many individuals would oppose testing treatments or drugs on animals, as they believe it is inhumane. Large opposition, especially after the involvement of beagle dogs and mice in investigations concerning the evidence linking smoking to disease, has led to the establishment of the 1986 Animal Act. This law launched a three-tier licensing system to limit and control animal experiments, as well as laying down ethical standards, which means that animal testing can only take place when there is no other alternative. On the other hand, experiments such as the one described above have indirectly prevented millions of premature deaths of humans.

Potential toxicity of nanoparticles raises concerns. For example nanoparticles used in cosmetics due to their small size might have an ability to penetrate through skin and interact with DNA in human skin cells with a potential to damage it. The safety of zinc oxide, widely used in sunscreens is being currently evaluated by the European Committee. With nanotechnology being a relatively new science there is still a limited understanding of direct and potential long term effects on human beings.

The use of nanotechnology, as a standard treatment, raises a possibility of dehumanisation of medicine, as mainly minute high technology devices would be the main constituent of treatment. The role of human beings as doctors, nurses and other medical staff could be minimised with increased dependency on technology. Vulnerable individuals, including the elderly and children, may not be comfortable and trustworthy of the high mechanical treatment, which they would find difficult to understand.

Moreover, there is a potential for unbalanced distribution of nanotechnology within a country and worldwide. There is a risk that nanotechnology will produce a ‘swapping body parts’ mechanism, in which only wealthy people will benefit. This will lead to a bigger difference in life expectancy between the affluent and the underprivileged. As a result, a bigger divide will be created in societies, with potentially only more economically developed countries (MEDCs) benefiting in the success of nanotechnology. A lack of clear ethical standards could mean that MEDCs could test their new developments on people from less economically developed countries (LEDCs). It also creates an essential question of whether the findings of nanotechnology in MEDCs should be shared with LEDCs to close the poverty gap. If this was the case, our population would increase even further due to increased life expectancy, which could have serious implications on our short supply of vital resources.

There is a need for internationally accepted standards in nanotechnology. Lack of such standards can lead to investment in nanotechnology in countries with weaker national regulations which then transfer the risks worldwide. That can lead to a displacement of jobs with impact on the economy, but more worryingly production of unsafe technologies and potential malicious or unwise use of nanotechnology. The International Risk Governance Council (IRGC) highlighted potential social and ethical issues including protecting the interests of those potentially affected by nanotechnology. The workshop highlighted a need to design inclusive, globally focused risk governance strategies [16].

Conclusion

The scientific field of nanotechnology can be used in a number of ways in regenerative medicine and can assist with treatment of strokes and CNS injuries. Application of nanotechnology in neuroregeneration can help to overcome factors limiting current treatments like the formation of scar tissue or limitations of crossing the blood-brain barrier. At present stem cells hold the greatest potential in regenerative medicine, however there are still several obstacles that must be overcome before their application can be defined as a success. The application of nanotechnology allows stem cells to reach the point where they are needed. Nanotechnology is also useful in a more direct form of treatment in the field of regenerative medicine. Carbon nanotubes are used as neural implant materials because of their conductive properties. Nanoparticles can provide targeted cell delivery of drugs to damaged nervous cells and improve the diagnostic process. Application of nanotechnology can also play an important role in neuroprotection and neurostimulation. Although there are currently a number of treatments that apply nanotechnology, many have not been explored in enough detail and many of them only tested on animals, which is why they are not regularly used. Further research into the field would be of significant benefit and would allow us, to use it in a safe and effective way to treat patients. Currently, passive and active nanostructures are being used, but according to the concept of "four generations" of nanotechnology development, presented earlier, further progress can lead to the development of molecular nano-systems. *"The power of these techniques is very impressive. This is clearly contemporary engineering. These technologies are no longer science fiction. It's fact"* (Prof Peter Agre). In the future, it is thought that neurosurgeons will be required to administer the nanotechnology based devices to the patient. Nanotechnology has a potential to revolutionise current treatments and reduce individual and social impact of CNS injuries and strokes.

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