NANOSTRUCTURED SCAFFOLDS AND STEM CELLS: THEIR POTENTIAL FOR USE IN TREATING SPINAL CORD INJURIES

BY
DANIEL CURLEY
VIVEK MAJUMDER
CIARAN MURPHY

PASS WITH DISTINCTION

RESEARCH PAPER
BASED ON
PATHOLOGY LECTURES
AT MEDLINK 2010

ABSTRACT
Spinal cord injury in humans remains a devastating and incurable disorder. The injuries often impair patients' movements by interrupting their motor-sensory pathways [6]. It is estimated that there are approximately 2.5 million people worldwide with a spinal cord injury, often bound to spend the rest of their lives in a wheelchair. After discovering the success of Professor John Kesslar at Northwestern University and his work on repairing broken spinal cords in mice [17], we undertook a Gedankenexperiment to reveal what this aspect of nanotechnology potentially has to offer for the estimated 2.5 million sufferers of a spinal cord injury, globally. As no clinical human trials having yet commenced, we do not fully understand whether this would work on humans and indeed do not understand any complications that may arise post treatment. The use of nanostructured scaffolding together with neural stem cells appears to be a promising route to repair of this traumatic injury. This approach may well cure paraplegias and quadriplegias, and possibly other types of paralysis in future generations.

INTRODUCTION

A man’s beard, on average, grows 5 nanometres every second. A nanometre is very small: 0.000000001 of a metre. Nanotechnology is the manipulation of matter at an atomic scale. Often hailed as a revolutionary new technology, nanotechnology has the potential to impact almost every area of society.

Twenty years ago, Don Eigler, a scientist working for IBM in California, wrote out the logo of his employer in letters made of individual atoms. This feat was a graphic symbol of the potential of the new field of nanotechnology, which promises to rebuild matter atom by atom, molecule by molecule, and to give us unprecedented power over the material world [9].

Nanotechnology is already in use in everyday products all around us, from windows to sunscreen, the use of tiny nano-sized particles has improved many mainstream products. Ingredients like zinc oxide can leave a white sheen behind, but sunscreens with zinc oxide nanoparticles rub on clear; the nanoparticles are too small to reflect light. Nanoparticles become energized when heated by UV radiation and begin to break down and loosen organic molecules (dirt) on Pilkington’s self cleaning glass [7]. It is expected that in the coming decades nanotechnologies such as improved electronics equipment, will be developed and have far-reaching implications.

In the realm of medicine, nanotechnology promises to provide great benefits for society in the future. Nanotechnology is already being used as the basis for new, more effective drug delivery systems and is in early stage development as scaffolding in nerve regeneration research. Furthermore, we will hopefully learn more about intervening in our biology at the sub-cellular level and this nano-medicine will give us new hope of overcoming really difficult and intractable diseases, such as Alzheimer's and Parkinson’s Disease, that will increasingly afflict our population as it ages.

Nanotechnology research with regards to cancer has seen much progress in recent times. The (American) National Cancer Institute has created the Alliance for Nanotechnology in Cancer in the hope that investments in this branch of nano-medicine could lead to breakthroughs in terms of detecting, diagnosing, and treating various forms of cancer. For example, a nanoparticle can be used as a targeting agent that can recognize a cancer cell and distinguish it from a healthy cell. By attaching a drug that actually kills cancer cells, you have a smart drug that knows which cells to attack and leaves normal cells unharmed. These new drug therapies have already been shown to cause fewer side effects and be more effective than traditional therapies.

Nanotechnology has many potential uses in nerve and cell regeneration; most notably its potential to assist in the repair of severed spinal cords via nanostructured scaffolds. Severed spinal cords and subsequently the loss of movement in limbs, is currently incurable. As aforementioned, a spinal cord injury normally results in a patient being wheelchair-bound for the rest of their life. Nanostructured scaffolds - a form of nanotechnology – has been proven to assist repair spinal cord injury in mice. When used in tandem with another emerging technology – stem cells, results looked extremely promising. It is this that we will discuss.

Stems cells are undifferentiated cells that can increasingly be manipulated by scientists. They do not have a specific gene expression [5] so they can differentiate into any type of cell of the species they belong to [1]. This is useful for modern medicine as it will provide many different treatments of various conditions in the near future. In November 2008 British doctors completed the first successful trachea transplant which was 'grown' *in vitro* in a laboratory at Bristol University [19]. The cells in the trachea had exactly the same DNA as the patient which is advantageous as it prevents an immune response against the organ which is much less costly than current methods or transplantation.

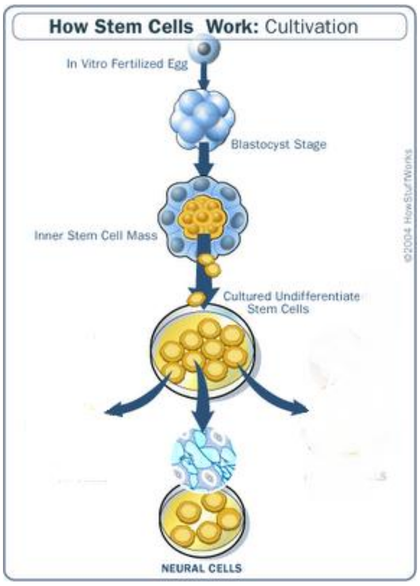

Fig 1.How stems cells work [11]

DICUSSION

Medical science today consists of many disciplines and concerns a number of different materials. These materials are currently being manipulated on an atomic level which will be vital for future medical advances. The use of these nanotechnologies could be helpful in treating and curing spinal cord injuries, a disease which disables millions of people world wide, and costs the NHS about £100m over one generation.

Research by Dr Fabrizio Gelain and his team at the university of Milan-Bicocca have led the field in nanostructured scaffolds made from self-assembling peptides and electrospun fibres which act as guidance channels. Their research focused on the repair of rats that have injured spinal cords; and it has proved that previously paraplegic mice regained a certain amount of control to their hind legs [3].

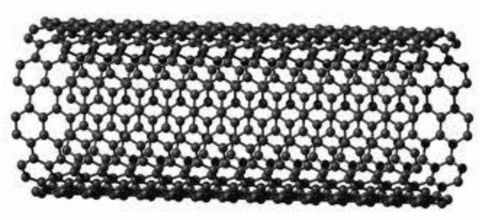

Fig (2) A nanotube scaffold structure [15]

Similarly, the work of Professor John Kesslar at Northwestern University and his team of researchers has proved that nano-engineered gel can be injected by an epidural at the site of injury within mice to again act as a scaffold around the injured cord encouraging repair [17].

By inserting molecules that are designed to self-assemble into nanostructures in the spinal tissue, they have been able to protect the spinal cord from the formation of scar tissue and cysts in acute injuries. It is this formation of scar tissue and cysts that prevent the spinal cord regenerating as the nerves cannot bridge the gap that the cysts cause in the injured, and they cannot penetrate through the scar tissue [23][14]. Also axons that are already present cannot produce a myelin sheath with scar tissue present. The myelin sheath protects the axons which are vital for the electrical impulses of the nerve [4][2]; this is the primary thesis of these new scaffolding technologies. The scaffolding enables the nerves of the spinal cord to repair naturally without the inhibition of any scar tissue or blocking syrinxes. Furthermore, the guidance channels proposed by Gelain bridge the gap caused by the formation of cysts.

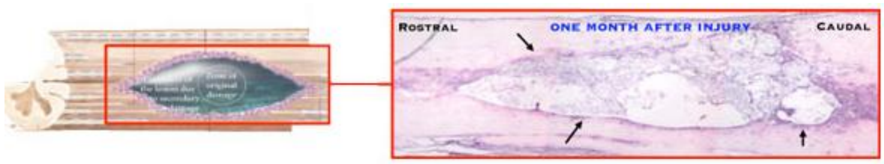

Fig 3. Syrinxes and scar tissue shown in acute spinal cord injuries.(k)

When nerves in the spinal cord are damaged, scar tissue is produced in the form of glial scar tissue. This is the natural healing response for a damaged spinal cord. However, there are many detrimental effects caused by glial scar tissue. Firstly it prevents neuronal regeneration due to growth-inhibitors being secreted from the scar tissue [14].Current methods are available for suppression of this tissue but there are no permanent cures and these methods have faults. The nanoscaffolds aim to prevent inhibition from scar tissue permanently.

However, nerves are one of the slowest repairing tissues currently known. They repair themselves at 3.5 cm a year [20][16]. This is obviously insufficient for any sufferer of spinal cord injuries. As stated by Gelain “By themselves, nanostructured scaffolds will probably not solve the problem of regenerating chronic or acute spinal cord injuries. However they will become a necessary component of an effective multi-disciplinary therapy in the near future." Therefore, would it not be quicker and easier to simply insert healthy neurons into the guidance channels? This invites other disciplines such as stem cell research to help find a complete treatment.

The manipulation of stem cells may allow doctors to grow genetically identical specified cells for a specific area of the body. This may prove useful in many areas of modern day medicine, especially in spinal injured patients, if used in conjunction with nanostructured scaffolds and guidance channels.

Future developments may see stem cells being used to aid repair of spinal cords. Stem cells can differentiate into neural stem cells *in vitro*. This should rapidly increase the speed of recovery as it means injecting the needed cells, already able to function, into an area that is needed to heal, whether this is a syrinx or a lesion. Stem cells would be obtained from the bone marrow, or for younger generations from a stem cell bank, which contains harvested stem cells from the respective e patient's umbilical cord. The use of stem cells for spinal cord injury has been proven to promote the recovery of rats with spinal cord injuries, but not actually cured the rats [12]. This has been shown by many researchers worldwide [12]**.** If they do heal it would take a long period of time as the stem cells would need to align themselves correctly in order to function properly. Also without direction, the stem cells might create a neuroma, causing ultra sensitivity and severe pain for the patient [21][13] .

<u>Method 1</u>

One possible future of spinal cord repair lies in both nanotechnology and stem cell technology, this treatment will combine both the nanostructured scaffold with the neural stem cells. This will achieve all the advantages of both the previously mentioned methods: the protection acquired from the scaffolding and the speed of recovery obtained from the insertion of neural stem cells directly into the point of injury; syrinx, lesion or other spinal injury. Additionally it would ensure that the stem cells are anchored (to the nanostructure) and therefore cannot 'freely float' away from the site of injury.

The method would see the insertion of a nanostructured scaffold around the point of injury as shown by both Gelain's and Kesslar's research. We would then inject the stem cells into the guidance fibres where they would be anchored to the scaffold and guided to the neurons. This is an *in vivo* implantation of the neural stem cells. Alternatively, one could anchor the stem cells to the scaffolds prior to insertion of the scaffolds. This is an *in vitro* approach to the treatment. Either method would theoretically obtain the same results however they both produce their own clinical and practical benefits and set backs. We assume that the *in vivo* method would be quicker and would require less expertise as both the scaffolding and the stem cells can be produced separately and merely injected into the body. As the guidance tubes are only one cell thick, the cells would automatically spread out in line with each other and be anchored to the nanoscaffolding. On the other hand, this method may be inaccurate and the stem cell injection may miss the guidance tubes and therefore be rendered useless.

Similarly we assume that the *in vitro* method will be a slow process as each stem cell would need to be aligned individually as the cells are attached to the nanostructure prior to insertion. This would require more expertise and would indeed be slower, however it is a more accurate approach as it is almost guaranteed that the stem cells will be anchored satisfactorily to the nanostructure and be in the correct place.

Either of these two methods obviously have huge potential applications in medical sciences and may very well be a cure for spinal cord injuries both chronic and acutely and perhaps similar conditions such as Parkinson's disease and Alzheimer's. This approach may well cure paraplegias and quadriplegias, possibly, other types of paralysis, enabling them to have functioning limbs. Furthermore, any breakage of the spinal cord above the diaphragm results in severe breathing difficulties and this new innovation may offer doctors another treatment.

Overall advantages of this proposed treatment may include: possible cures for current incurable conditions, quick relief for acute injuries, enabling current paraplegics or quadriplegics to use their affected limbs again, potentially free up bed space in hospitals, possibly reduce costs of sustaining chronically impaired patients both in hospital and domestically and reduces waiting times for patients.
These advantages should occur as this process would be relatively quicker than current known methods and offer regeneration of neurons, hence spinal cord repair.

On the other hand, there are also many disadvantages of this proposed treatment. The main disadvantage of this idea is that only the separate methods (nanostructured scaffolding and stem cells) have been proven to work in mice and rats alone [8]. No clinical human trials have yet commenced therefore we do not fully understand whether this would work on humans and, indeed, do not understand any complications that may arise post treatment. Furthermore we can only assume that the two technologies will work together. Supplementary disadvantages include: the cost of these technologies may well be extravagant, any ethical issues from animal testing as well as the use of stem cells and we do not fully understand any mutations that may occur after the treatment.

Method 2

Similarly, nanostructured scaffolds could theoretically be used in union with stem cells to allow doctors to perform a bypass of the spinal cord. This would achieve the same outcome as an arterial bypass which are commonly used by vascular surgeons to 'bypass' the blocked or damaged section of the artery under treatment [22].

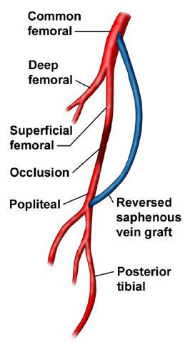

Fig (4) An arterial bypass [10]

The proposed treatment would bypass the damaged area of the spinal cord as glial scar tissue or cysts may be present. The nanoscaffold would be inserted parallel to the site of injury and connected to both the functioning neurons at either end of the injury. The addition of stem cells into the scaffold would promote new healthy neurons to grow and potentially repair. This is because the peripheral nerves can be repaired. Further studies may well see 'nerve grafts' with the use of nanotechnology. This would involve a similar operation but surgeons would take a healthy nerve from the chest for example and insert it into the spinal column. The graft has already been used on an American man who reported that he could move his legs, albeit not by a huge amount [18]. The introduction of nanoscaffolds may well be used with this treatment in the future and offer long term benefits for patients.

Ethics

There are a multitude of ethical issues that we would face in both the research and the implementation of these treatments. Firstly, as no human tests have currently been done, we do not know the effects of the materials (such as proteins) used in the nanostructured scaffolding. These may lead to rejection and may be targeted by lymphatic cells or be poisonous to the body. If this is the case, then the treatment will be deemed ineffective. The proteins may be mutagenic or carcinogenic to the neurons and neural stem cells. If in the case of it being mutagenic, the antigens of the cell might change so this may cause an antigenic immune response to the actual neurons of the spinal cord furthering the injury. If they are carcinogenic they would cause a cancer of the spinal cord which may very well spread throughout the body.

Further ethical issues include the use of animals in testing, which has been much debated since it commenced. The research carried out by Gelain and Kesslar both used a weight dropping device to break the spinal cord of the rats and mice respectively. Many critics condemn this and brand it unnecessary cruelty to animals as they believe that animals should be treated with the same respect as humans. However, complying by the UK's Animal Scientific Procedures Act 1986 - and similar acts around the world - researchers cannot be prosecuted for the use of animals in their experiments.

Finally, the major ethical issue presented by sceptics is the use of stem cells in particular, the use of human embryos acquired from *in vitro* fertilisation for research on stem cells. This is because embryos are a highly rich source of stem cells as the cells are in an early stage of life and so they are not yet differentiated. Many critics believe that this is an act of murder as they believe that the embryos are already human beings and have all the same rights as a fully developed human being. Furthermore, many believe that these advances in science may be classed as 'interfering with nature' or 'playing God' as you are growing new organs or cells. However these are deemed legal by the UK Stem Cell Research Enhancement act 2005. However this may not be the same worldwide as many countries are still sceptical and religiously bound against it.

CONCLUSION

Spinal injury is devastating, costly to manage and resistant to standard treatments. The introduction of nanotechnology offers theoretical opportunities for research into and treatment of these challenging conditions. Conceptually, the combination of physical structures – nanoscaffolds – lined by stem cells could have significant beneficial roles in management of spinal injury.

Previewing rodent research into spinal injury suggests that human trails may be valuable. However, whilst this science has many supporters, it remains controversial and is strongly opposed by some on ethical and religious grounds. Careful examination of these combined technologies may offer hope to a devastating condition.

Bibliography

1. Bruce Alberts, Dennis Bray, Julian Lewis, Martin Raff, Keith Roberts, James.D.Watson (1993) Molecular Biology of the Cell, Page 910-912

2. Ambrose, Easty (1977) Cengage Learning Australia, Cell Biology, Page 158

3. Michael Berger, Nanostructured scaffolds offer a promising route to repairing spinal cord injuries: http://www.nanowerk.com/spotlight/spotid=19962.php

4. Biofactsheet Number 20 (1998), Nerves and Synapses

5. Biofactsheet Number 45 (1999), Gene Expression

6. Biofactsheet Number 56 (1999), Autonomic Nervous System

7. Current, everyday uses of nano-particles: http://science.howstuffworks.com/nanotechnology3.htm

8. Embryo stem cells help treat paralysed rats: http://www.bionews.org.uk/page_12353.asp

9. Guardian's 25 predictions for the future: http://www.guardian.co.uk/society/2011/jan/02/25-predictions-25-years

10. Image of arterial bypass: http://yoursurgery.com/ProcedureDetails.cfm?BR=5&Proc=33

11. Image of 'how stem cells work': http://www.boncherry.com/blog/2010/02/20/stem-cell-banks-fraud-warning/

12. John W. McDonald, Xiao-Zhong Liu, Yun Qu, Su Liu, Shannon K. Mickey, Dennis W. Choi (1999) Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat's spinal cord

13. Medical Dictionary Saunders company 23rd Edition p482, 483, 662

14. The Merck Manual 14th Edition 1337-1373

15. Nanotubes and fullerenes for Quantum Computing: http://homepage.mac.com/jhgowen/research/nanotube_page/nanotubes.html

16. Nerves & Vascular Injuries of the Hand: http://www.practicalplasticsurgery.org/docs/Practical_33.pdf

17. Northwestern University (2008) Promising new nanotechnology for spinal cord injury: www.sciencedaily.com/releases/2008/04/080402114819.htm

18. Spinal Cord Bypass Surgery “Breathes” New Life into Man’s Legs: http://www.columbianeurosurgery.org/2010/02/spinal-cord-bypass-surgery-breathes-new-life-into-mans-legs/

19. Trachea Transplant: www.thetelegraph.co.uk/health/healthnews/347913/British-doctors-health-preform-worlds-first-transplant-of-a-whole-organ-grown-in-lab.htm

20. Verbal Communication: Mr S Majumder (Consultant Plastic Surgeon)

21. Verbal Communication: Dr P Manraj (Consultant Anaesthetist)

22. Verbal Communication: Mr P Curley (Consultant Vascular & General Surgeon)

23. Williams & Warrick (1993), Gray's Anatomy, 36th edition, page 864 – 869