

**Future prospects of the Treatment of
Traumatic Brain Injury
through developments in Nanomedicine**

BY

GETHIN HOPKIN

ALICE JUDGE

PASS

(120 words over limit)

RESEARCH PAPER
BASED ON
PATHOLOGY LECTURES
AT MEDLINK/VET-MEDLINK 2010
Word Count: 3,980 words

Abstract

Nanotechnology is currently being developed in many areas of medicine. The ability to manipulate atoms on such a small scale opens up a world of possibilities in cell repair, diagnosis, treatments of cancer and alternative therapies. The idea of self assembling molecules has recently been researched (machines or structures that assemble themselves into a specific design.) This paper explores the potential of SAPNs (Self Assembling Peptide Nanostructures) in the treatment of brain injuries. These structures – when activated by ionic solutions can form ‘scaffolding’ for new neurons to form. As currently there is no real effective treatment for TBI patients except preventing deterioration of the brain and trying to facilitate new growth of axons the implications of SAPNs are huge. They present a completely new approach to treatment of TBI patients - rebuilding the neurons in the brain. Problems arise when considering delivery of the SAPNs to the specific site of damage; however with current developments it may be possible to use engineered Nanoparticles or artificial liposomes to deliver them.

Introduction

Nanotechnology is the study of manipulating structures and properties on an atomic level, working on a scale of about 1 – 100 Nanometres. This new technology has huge potential for the improvement in health and medicine as well as a number of developments in physics. What makes this technology so distinct and different is its fundamental building blocks are atoms or molecules - unlike current devices that are miniaturised versions of macroscopic machines.¹

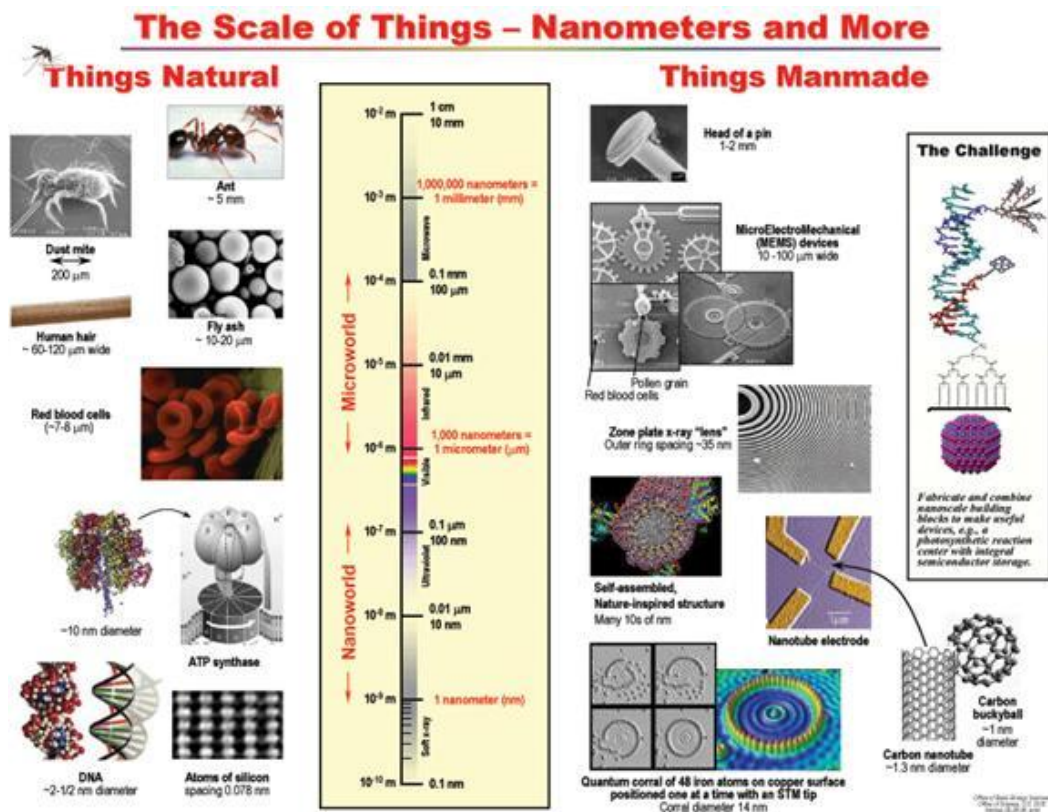


Figure 1: Demonstration of relative sizes in science

There are two general approaches: ‘top – down’ where larger materials and devices are downsized to Nanoscale to create Nano-objects, and ‘bottom – up’ where molecular devices are composed using atoms or molecules as building blocks on an atomic level. Because of their tiny size, Nanomaterials have special properties that make them ideal for a range of commercial and medical uses.²

There are a multitude of possible applications of Nanotechnology in medicine, many are undergoing research currently and new ideas are still being thought up as it is a very new concept to the entire scientific community. Applications of Nanotechnology for treatment, diagnosis, cell repair, monitoring, and control of biological systems has recently been referred to as ‘Nanomedicine’. It is already forming a basis for new, more effective drug delivery systems and nerve regeneration research. There are also hopes that Nanomedicine could lead to breakthroughs in detecting, diagnosing and treating various forms of cancer. These molecular machines or engineered Nanoparticles can be used to deliver pharmaceutical, therapeutic and diagnostic agents to specifically targeted cells or receptors. This targeted drug delivery limits damage to healthy cells, as can be experienced majorly in chemotherapy.³

Nanoparticles themselves have a wide variety of uses in the field of medicine. Research currently underway includes Aluminosilicate Nanoparticles in trauma patients⁴. These particles absorb water, causing blood to clot quicker. Z-medica is producing a medical gauze that makes use of Aluminosilicate’s useful properties. Nanobiotix has recently released results on an alternative technique to radiation therapy⁵. The Nanoparticles attach themselves to cancerous cells, then release electrons when activated by X-rays which lead to the destruction of the cancerous cells.

One of the most contentious issues in the field of Nanotechnology is that of Molecular Assembly, the idea that atoms could be arranged so precisely as to facilitate highly precise reactions according to specific requirements⁶. Much money has been spent on research in the area, and much time spent by leading Scientists such as Dr Kim Eric Drexler and Nobel Prize winner Dr Richard Smalley debating the issue. In fact, the idea of such ‘Nano-factories’, or other self-replicating machines on an atomic scale has caused much controversy (caused by ideas like the “grey goo” theory) around the whole field of Nanotechnology, leading to many ethical and practical problems preventing Nanotechnology being allowed to reach its full potential. There is, however, a field within Nanotechnology which research has shown to be both safe, efficient, and one which could have major implications in Medicine, that of Self Assembling Peptide Nanostructures (SAPNs). Although these new technologies have implications across the whole of Medicine, an area in which its implementation would have a major effect would be that of Traumatic Brain Injury (TBI).

Discussion

Traumatic Brain Injury Mechanisms

It is firstly important to explain the mechanisms which cause TBI, before exploring how the implementation of SAPN could have an impact on the treatment of this injury.

TBI is the medical name for what many in the public know as ‘brain damage’, and it has both many causes, and many forms in which the damage can occur. There are around 1 million cases of TBI annually in the UK alone, and one of the most common causes of TBI in not only the UK, but internationally, is Road Traffic Collisions (RTCs), thus this section uses an RTC to explore examples of the mechanisms behind TBI. In a large number of cases where TBI occurs in RTCs the fast movement on impact alone will lead to damage. This is due to the fact that the head moves in one direction, and as the brain is ‘suspended’ inside the cranium, it does not move at the same time, and collide with the posterior internal surface of the cranium. In most cases where this type of injury occurs, the damage is likely to be mild, as the momentum of the head is lost gradually, as opposed to the sudden stop involved in collision of the head with a solid object. The second type of TBI caused in RTCs is likely to lead to more severe damage, in the instances where the head’s movement is stopped by sudden impact with a solid object – most often a dashboard or seat in front. The same principle is observed as above, but the sudden loss of momentum of the cranium on impact leads to the ‘suspended’ brain losing its own momentum on collision

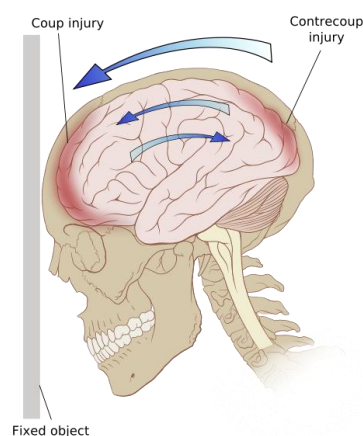


Figure 2: Illustrates the coup-contrecoup phenomenon

with the anterior internal surface of the cranium at high speeds. A third type of TBI is caused mainly in instances of sudden collision as highlighted in the second example, and is known as the ‘coup-contrecoup phenomenon’. This type of TBI mechanism is caused when the sudden impact causes the brain to ricochet within the cranium, damaging both the anterior (coup injury) and posterior (contrecoup injury) surfaces of the brain.^{7,8} This paper deals with instances of impact TBI not piercing TBI, as the SAPNs discussed later have more effect on the former.

Traumatic brain injury pathology

There are two main types of lesion to deal with in this paper, focal – specific to one area, and diffuse – generalised spread of injury across numerous areas.⁹

One of the most obvious, and potentially most dangerous in the short-term, injuries caused to the brain is the haemorrhaging of blood vessels within the brain. These tend to occur focally if caused by a piercing injury, but can also occur for biological reasons, such as a stroke, or a burst aneurism in a cerebral blood vessel. It cannot be emphasised enough how damaging haemorrhages in the brain are, and in cases of intra-axial (in-brain) haemorrhage, 34-50% of patients will die within 30 days.¹⁰ Diffuse Lesions can be microscopic, and were, until the

decade of the 2000s, only readily detected under microscopic examination during a post-mortem. In the 2000s, however, a technique called Diffusion Tensor Imaging was developed, which measures diffusion of water in the white matter of the brain - thus allowing the detection of extremely small but widespread damage.¹¹

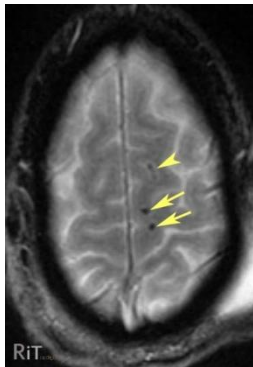


Figure 3: MRI scan indicating axonal lesions

On impact, the neurons of the brain themselves, not just simply the blood vessels, can be severely damaged. Under the extreme forces of acceleration and deceleration experienced in incidences of TBI, different areas of the brain which have different masses and densities will travel at different speeds. This stress on the neurons can lead to stretching, disfiguring, and commonly sheering of axons (connective branches within neurons which carry electrical impulses).⁷ The axons are absolutely vital to the function of the brain, as they form the white

matter tracts along which electrical transmissions are carried, leading to specific bodily and psychological functions. Thus it is extremely damaging when axons are damaged, and when diffuse axonal injury (DAI) occurs, it is highly common for patients to go into comas, or deep levels of unconsciousness. In instances of DAI sufferers going into comas, it has been estimated that at least 90% of patients will go into a permanent vegetative state.¹²

The implications of these injuries are, in the first instance, that unless urgent and effective treatment are sought, the worst case scenario is almost always death – by extreme intracranial pressure, blood loss, or damage to vital parts of the brain responsible for vital functions. In the longer term, the main problems relate to both physical ability and psychological issues. In a very high number of cases, sufferers of TBI report major behavioural changes, with a high number of people later developing recognised psychological conditions, or undergoing periods of unusual psychological behaviour.¹³

Implications of SAPNs for the treatment of TBI

Throughout the human system, there are many examples of chemicals which ‘self-assemble’ to form larger structures. Whether it is amino acids which form long polypeptide chains under condensation, or the assembly of nucleotides into DNA, our bodies are excellent at building from atom upwards to tissue and beyond, yet medicine relies, for the most part, on using comparatively large structures for treatment. In the case of TBI, the main course of treatment currently is simply to, in the first instance, prevent further deterioration of the brain, and secondarily the main treatment takes the form of rehabilitation (both physical and cognitive). There is currently very little which medicine can actually do to help the axons of the neurons damaged within the brain to recover, other than facilitate new growth through activity, and attempt to recover functions lost due to TBI. This is where the very important implications of SAPNs have the potential to help.¹⁴

SAPNs themselves are based on fairly elementary scientific principles, which work fairly simply in the human body. The most basic building block in the process is the L-amino acid peptide chain, of alternating positive and negative amino acids, of approximately 5nm in length. On assembly, the peptides form slightly larger chains of approximately 10nm long, which themselves then go on to form significant areas of ‘scaffolding’. This process is initiated when SAPNs are introduced into ionic solutions. When experimentation on SAPNs is carried out in vitro, the easiest way for scientists to cause this self assembly is the use of a saline fluid, which is highly ionic. This is why the process is so simple in the human brain - cerebrospinal fluid (CSF), which occupies a significant volume of space around, and inside the ventricles of the brain, is itself ionic.¹⁵ Effectively this mimics the saline solution used in vitro, thus the introduction of SAPNs to CSF in vivo triggers the assembly process. It is also important to note that the area of scaffold produced is directly proportionate to the concentration of peptide in the solution used – this means that in an application of SAPNs in humans, the area of scaffold produced could be controlled to a degree.¹⁶

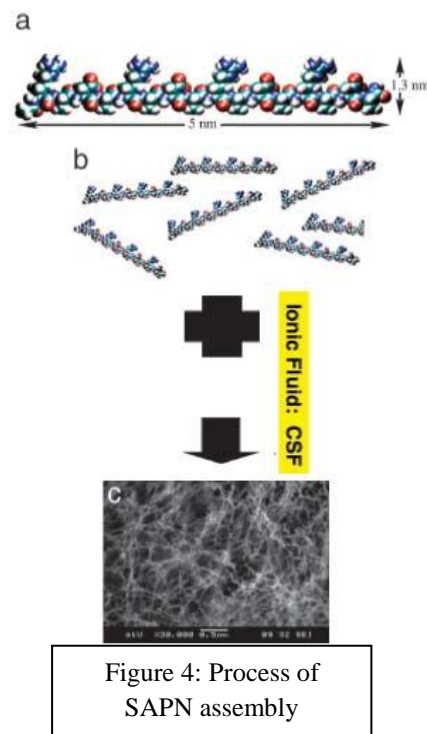


Figure 4: Process of SAPN assembly

There are a number of obstacles to overcome in the process of recovery from DAI, and possibly the most significant of these is the fact that during TBI, both laceration of tissue, and the phagocytosis of dead cells, cause lesions (as discussed above) which can prevent regeneration of neurones.¹⁷ The neurons themselves are only able to regenerate their axons to a fairly limited length, which can make it very difficult for the neurons to cross the lesions in the brain.¹⁸ This is where SAPNs can have a major impact. The neurons may not be able to bridge the lesions in the brains of their own accord, but the scaffold created by SAPNs can provide a network of structures within a lesion which facilitate the regeneration of neuronal axons. The neurons can grow across the gaps using the scaffold as a point of contact from which regeneration can occur. Instead of having to bridge the relatively large gaps presented by lesions, the neurons only have to bridge the gap small section by small section by utilising the scaffold.¹⁹

The introduction of SAPNs into lesions

Although the science is behind, and practical applications of (in vitro) SAPNs are well documented, there is still one fundamental question which confronts scientists and medical professionals if SAPNs are ever to be used in the treatment of TBI sufferers:

- “how do we get the SAPNs into the brain in the first place?”-

There are two potential answers which provide the best treatment, but each one has limitations attached to it, so I will explore these individually.

Direct application into lesion

This process would involve the direct introduction of the SAPNs via a solution into the lesion itself. This would probably be deemed the most effective approach, as a very precise concentration of solution could be very precisely delivered accurately into the required area. The other major advantage of this approach is quite simply that it would cut down the time taken for repair to begin, as the scaffolds would form very quickly, as no transport to the site of lesion would be required. There is, of course, one very major limitation to this method: the simple fact that direct access to the lesion would have to be gained, whether this be through some form of cranial laparoscopic method, or by actually opening up the cranium itself as if for a surgical procedure. Of course this is not likely to be a problem for lesions arising from haemorrhages or lacerations which have incurred surgery anyway as the cranium is already open, however the problem arises when using SAPNs to treat DIA. Because of the nature of DIA it would be difficult to isolate precise spots for delivery of SAPNs, and so the direct application would be highly impractical and inefficient in the treatment of DIA. However, it still remains a feasible option for treatment where large isolated lesions occur, as detected on MRI scans or similar.

Intra-venous (IV) delivery

As one of the most effective methods of non-specific drug delivery in many medical situations, one would logically look at the option of IV delivery for introduction of SAPNs to lesions. The key area where these could benefit would be the treatment of DIA, where many microscopic areas of axonal damage could be reached effectively by SAPNs being distributed in the blood stream. Set concentrations of SAPNs could be injected very easily by a range of medical practitioners, very early on in the development of DIA, giving the patient the best chance of making a recovery.

Two significant problems stand in the way of this method:

The majority of IV drugs used in medicine today are, although not necessarily specific, likely only to interact with the targeted area, or at least have limited side effects on other areas. SAPNs start assembling, however, as soon as they are introduced to an ionic fluid. Thus on injection into the blood stream, when the SAPNs are distributed throughout the body, the scaffolds would start to develop in practically every area the solution has spread to. This is a major obstacle, and one which could completely rule out IV delivery unless a suitable resolution is found to the problem.

A second practical obstacle to the use of IV delivery is that even if a way of controlling the SAPNs so that they do not prematurely assemble was found, then it would be very difficult to get the SAPNs across the Blood-Brain Barrier (BBB). The BBB is effectively a safeguard in the brain which prevents certain solutes, and things such as bacteria, from crossing from the blood stream (in the form of capillaries in the brain) into the CSF. This safeguard regulates the chemical composition of the CSF, and, when working healthily, prevents infections from getting to the brain. Even if the SAPNs reached the blood vessels in the brain, they would not

be allowed to cross over the barrier into the CSF, and so could not assemble into the scaffolds useful for axonal repair.

There could, however, be a solution on the horizon for overcoming the BBB for the purposes of SAPNs delivery – the use of liposomes to cross the barrier. These artificial carriers are made by artificially constructing the structures from the lipid bilayers found in the plasma membranes of eukaryotic cells. Current research focuses mainly on the use of these Nanoparticles for targeted drug delivery to brain tumours, but I believe that these could be influential in the delivery of SAPNs. There is one limitation in this practice, which is that each liposome would have to be large enough to contain the SAPNs for transportation. For liposomes to cross the BBB they must be of a relatively small size, and so could only carry an extremely limited number of SAPNs over the barrier each. If, though, money and time were to be invested in the usage of liposomes to cross the barrier, I believe that a potential solution could be found, which would be very promising to the field of SAPNs treatment in TBI patients.

The logistical applications

It is here that I propose the much more theoretical future of the application of SAPNs which has been given almost no public discussion previously. I propose the, some would say radical, but I would say feasible, once (and this is provisional of effective and safe clinical trials, followed by NICE approval) SAPNs are available for TBI treatment, a programme be rolled out across the country for their implementation. Considering the vastly improved technology used for detection of DIA as discussed above using DTI, and the greater

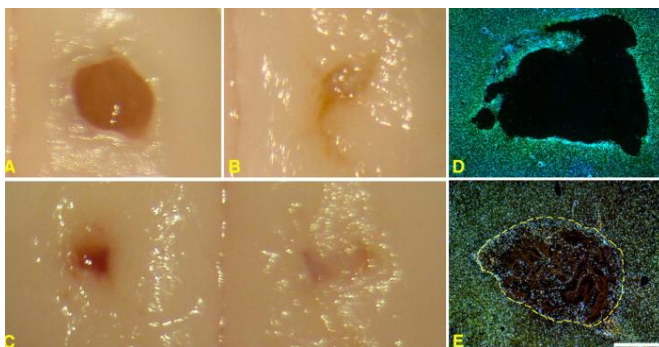


Figure 5: Top row, lesions before treatment.
Bottom row, lesions after treatment with
SAPNs (in rat brains)¹⁹

availability of this technology, I believe there is scope for the use of SAPNs to be applied in a much greater number of units which treat TBI patients.

It is, of course, very difficult to realistically discuss the idea of rolling out a programme of SAPNs without mentioning two things. Firstly, the research which looks likely to continue in the future is still going to be limited only to animals, and it is likely to be many years until the research scientists

involved with their development are likely to be in a position to move forward with testing the technology in humans – let alone seeking governmental approval for clinical trials. The second issue relates purely to finance – we have literally no idea as yet of how much SAPNs will cost to manufacture, distribute, and implement. It is for this reason that, even after provisional NICE approval, SAPNs are likely to face staunch opposition from many sides. Cost is always an issue in medicine, and with politicisation of issues such as medical technology ever present, would SAPNs struggle to enter mainstream treatment? I think not. It has been seen in many cases that when technology initially arises, and cost seems a major

disadvantage, people do eventually see the benefits of its implications, and are supportive of its introduction.

My suggestion; that wherever a unit is present to deal with TBI instances, the facilities for SAPNs treatment should be available. Although, as mentioned, no studies have been carried out on human subjects, it would be very much logical to presume that the sooner SAPNs could be introduced to lesions, the sooner neurons could start to repair – thus the damage from TBI would be reduced greatly. Therefore it is also logical to presume that were SAPN facilities to be introduced into TBI treatment units, the damage caused to hundreds of thousands of sufferers of TBI in the UK alone could be greatly reduced. It is of course important to clarify what sort of places I advocate these facilities being introduced, but in fact it is a difficult thing to do, as so many places in the NHS are used for TBI treatment – whether it be rehab centres responsible for long term treatment, or the front line primary care services of the Ambulances and A&E departments of the country. It is here that we must re-examine the methods by which SAPNs could be introduced to lesions as discussed earlier. Unless facilities were only to be introduced into units which already coped with incidences of neurosurgery, it would be very difficult to apply the direct application to lesions method in real life practical situations. *This* is where IV methods take precedence. A wide range of practitioners, whether they be Nurses, Doctors or Paramedics, are currently very experienced and able at delivering drugs through intravenous therapy. If, and admittedly as earlier highlighted it is a very big *if*, a solution to the problems of IV introduction of SAPNs could be found, then it would not be that difficult for currently IV trained practitioners to be trained in the new treatment.

Ethically, there is not much to stop SAPNs from being further developed. They only mimic the natural processes which go on in the human body every day in protein development, and when broken down, reform into their natural amino acids which can be utilised by the body. Further to this, other than the anti-animal experimentation arguments which face many medical practices, there are no ethical problems similar to those confronting research in areas such as Stem Cells which SAPNs would face, simply on the basis that SAPNs are only a manipulation of naturally occurring compounds in the body, and not manipulation of living subjects of any form, or the utilisation of parts of living subjects.

Conclusion

It is very difficult to evaluate how useful SAPNs could prove to be in the treatment of TBI without actually testing the technology on the condition. For the meantime, it is likely that researchers will have to continue to test the idea on animal subjects. Which obviously raises ethical issues concerning animal rights and welfare; however, it is heartening to know that scientists are indeed having great success in the use of SAPNs in animals, and this bodes well for the future of the technology²⁰. There are, as discussed, some major problems which face the use of SAPNs in patients. Both methods of delivery present problems which would be very difficult to overcome. The direct application method does, out of the two, seem to present less of a problem. The issue is, however, that it would be difficult under this method to use SAPNs to treat TBI patients without neurosurgeons present. Thus limiting its applications drastically, as opposed to the much more straightforward IV method, which itself has flaws which would be extremely difficult to overcome. But they are something which should be looked at as separate issues, so as not to hinder the progression of research into the area. Future developments into specially engineered Nanoparticles for drug delivery may form a possible solution to delivering the SAPNs.²¹ It might be possible to combine Nanoparticles with monoclonal antibodies which could then target and deliver SAPNs to specific areas of the brain such as the lesions. Or, as discussed above, engineer artificial liposomes to carry the SAPNs across the Blood-Brain Barrier – which would overcome a big problem presented by the IV method. Also there are wider problems for any advances in Nanotechnology at the moment, including the issues related to toxicity and environmental impact of nanoproducts, as well as health risks and side effects of nanomaterials which are still not thoroughly investigated. Some critics question ‘who gets to decide what our future world looks like?’ and ‘whose interests are they being developed in?’ Nevertheless, the potential of Nanotechnology is a very exciting prospect and could perhaps one day present a life changing treatment for TBI patients world wide.

References

- 1 - <http://www.crnano.org/whatis.htm>
- 2 - <http://www.csa.com/discoveryguides/nano/overview.php>
- 3 - <http://pubs.rsc.org/en/Content/ArticleLanding/2009/JM/b902711b>
- 4 - <http://www.understandingnano.com/medicine.html>
- 5 - <http://www.nanobiotix.com/technology-products/>
- 6 - <http://www.imm.org/research/parts/controller/>
- 7 - <http://www.braininjury.com/injured.html>
- 8 - <http://www.ninds.nih.gov/disorders/tbi/tbi.htm>
- 9 - <http://www.ncbi.nlm.nih.gov/pubmed/8445204>
- 10 - <http://emedicine.medscape.com/article/1163977-overview>
- 11 - Denis Le Bihan, MD, PhD et al (2001) Journal of magnetic resonance imaging
13:534-546
- 12 - <http://emedicine.medscape.com/article/339912-overview>
- 13 - http://www.ninds.nih.gov/disorders/tbi/detail_tbi.htm
- 14 - <http://www.brainandspinalcord.org/traumatic-brain-injury-types/diffuse-axonal-injury/index.html>
- 15 - <http://ajpheart.physiology.org/content/254/2/H250.abstract>
- 16 - <http://www.innovitaresearch.org/news/06102501.html>
- 17 - <http://bmb.oxfordjournals.org/content/53/3/491.full.pdf>
- 18 - <http://www.neuropathologyweb.org/chapter4/chapter4aSubduralepidural.html>
- 19 - (Sept 2009) Nanomedicine: Nanotechnology, Biology and Medicine
Volume 5, Issue 3, Pages 345-351
- 20 - (Dec 2007) Nanomedicine: Nanotechnology, Biology and Medicine, Volume 3, Issue 4,
Pages 311-321
- 21 - (Dec 2009) Nanomedicine: Nanotechnology, Biology and Medicine, Volume 5, Issue 4,
Pages 369-377