

A Discussion of the Potential for the  
Use of Nanotechnology to Combat the  
Degenerative Effects of Alzheimer's Disease

By

Tomas Ellis  
Rosie-Loup James

PASS WITH MERIT

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### Abstract

In 1959 Richard Feynman first presented the idea of manipulating matter on the molecular level, allowing many further technological advances from other great scientific minds such as Eric Drexler, the author of *'Engines of Creation'*, Harold Kroto, Robert Curl and Richard Smalley, who all played a part in the discovery of Buckminsterfullerene (<http://www.crnano.org/whatis.htm>). These academic discoveries led to new medical advances in diagnostics and treatment, one such progression is in the field of drug delivery, and it is this that shall be the main focal point of the following paper. We will be exploring the possibilities of using nanotechnology in drug delivery systems, in order to diagnose and treat the effects of Alzheimer's disease. After extensive research and study in this area, we have come to the conclusion that a combination of Nano-technological techniques and specialised drugs would be both plausible and effective in the identification and treatment of Alzheimer's disease.

### Introduction

In recent years, there have been many developments in the field of nanotechnology, which have benefited our everyday lives. It has also opened up possibilities that a decade ago would not have been achievable and developments within the field of nanotechnology are constantly moving forward. The National Nanotechnology Initiative (NNI) (cited by McNeil, 2005, p.585) defines nanotechnology as 'research and technology development at the atomic, molecular, or macromolecular scale, leading to the controlled creation and use of structures, devices, and systems with a length scale of 1-100 nanometres.' Therefore, nanotechnology is the principle of manipulating atomic-sized structures, in order to produce more complex configurations, thus using nature itself as the inspiration. Nature's influence is evident in the scientific approach which has utilised the "bottom up" method present in living organisms, for example the combining of DNA molecules to form a chromosome, which then codes for all the characteristics of a human body (<http://www.crnano.org/whatis.htm>). If the DNA molecules were to be manipulated into a different sequence, the entire genetic make-up of the individual would alter, and this is the basic principle behind nanotechnology. Nanotechnology is particularly useful within the field of medicine as illnesses affecting the body, work on a cellular level, and nanotechnology has the ability to penetrate through the gaps in the phospholipid-bilayer of the cell membrane, allowing for more effective drug release. During the course of nanotechnological exploration, many theoretical advances have been made based on nature's model, and they are now beginning to make the leap into practical application.

Buckminsterfullerene for example, has specific chemical properties that allow it to be extremely useful in all walks of life. It can conduct like a normal metal, super conduct, act as an insulator, and change state at any one point in time. Not only this, but its ability to wrap itself around individual atoms and alter DNA and some sugars has the potential to change the way in which medicine is administered. Currently, a Buckyball-based Acquired Immune Deficiency Syndrome (AIDS) treatment is in early-clinical trials, as a fullerene-based AIDS drug has been discovered to work against all the drug-resistant HIV strains on which it has been tested so far. The idea of this C-60 drug is that the Buckminsterfullerene balls inhibit the production of an enzyme caused by the AIDS virus, known as HIV protease, by

fitting itself tightly around the active site of the enzyme ([http://www.org-chem.org/yuuki/C60/C60\\_en.html](http://www.org-chem.org/yuuki/C60/C60_en.html)). As shown in figure 1.

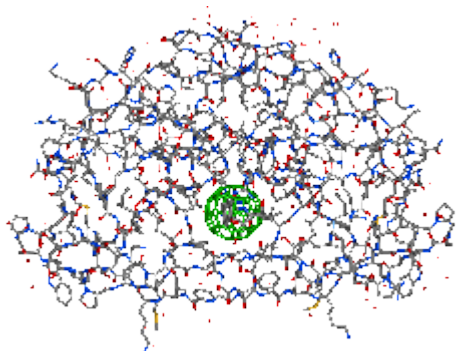


Figure 1 – A C<sub>60</sub> molecule inhibiting HIV protease  
([http://www.org-chem.org/yuuki/C60/C60\\_en.html](http://www.org-chem.org/yuuki/C60/C60_en.html))

Another nanotechnology that is becoming prevalent is that of the nanotube. Discovered in 1991 by S. Iijima, these extremely strong, long, thin, hollow cylinders of carbon atoms (approximately x10, 000 smaller in diameter than a single human hair) have become the focus of many research projects in the past decade, as their physical properties, molecular mechanics, and the manipulation of individual nanotubes to perform certain functions provide a host of possibilities for enriching human life. This ability to adjust the nanotube structure slightly in order to produce a specific desired physical property is especially useful; it has led to advances such as nanotube fibres (Figure 2) and nanoelectronics due to their ability to conduct when in certain structures (<http://www.research.ibm.com/nanoscience/nanotubes.html>).

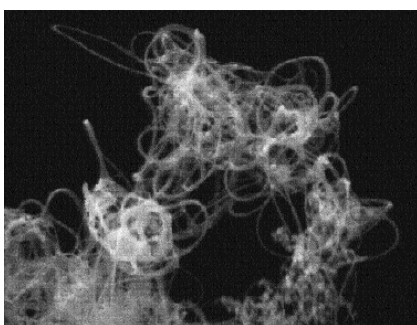


Figure 2 – Nanotube fibres will soon replace carbon fibres in everyday use  
(Andrews, 2001, <http://www.chm.bris.ac.uk/webprojects2001/andrews/nanotubes-uses.html>)

In medicine nanotubes can be used as biosensors, by again changing the structure slightly in order for it to detect many different molecules found in the blood. For example, loading a glass capillary tube with glucose-sensitive nanotubes, and inserting it into a blood vessel, will allow diabetics to monitor their blood glucose levels without the need for an

injection, by instead detecting blood glucose levels through the intensity of the fluorescent light emitted when an infrared light is shined on to it (Strano, 2004, <http://www.sciencedaily.com/releases/2004/12/041214081957.htm>). Figure 3 below sums up the current and future uses of nanotechnology.

Material/technique	Property	Applications	Timescale (to market launch)
Drug delivery			
Nanoparticles in the range of 50–100 nm.	Larger particles cannot enter tumour pores while nanoparticles can easily move into a tumour.	Cancer treatment.	?
Nanosizing in the range of 100–200 nm.	Low solubility.	More effective treatment with existing drugs.	?
Polymers.	These molecules can be engineered to a high degree of accuracy.	Nanobiological drug carrying devices.	?
Ligands on a nanoparticle surface.	These molecules can be engineered to a high degree of accuracy.	The ligand target receptors can recognise damaged tissue, attach to it and release a therapeutic drug.	?
Nanocapsules.	Evading body's immune system whilst directing a therapeutic agent to the desired site.	A Buckyball-based AIDS treatment is just about to enter clinical trials.	Early clinical.
Increased particle adhesion.	Degree of localised drug retention increased.	Slow drug release.	?

Nanoporous materials.	Evading body's immune system whilst directing a therapeutic agent to the desired site.	When coupled to sensors, drug-delivering implants could be developed.	Pre-clinical: an insulin- delivery system is being tested in mice.
'Pharmacy-on-a-chip'	Monitor conditions and act as an artificial means of regulating and maintaining the body's own hormonal balance.	E.g. diabetes treatment.	More distant than 'lab-on- a-chip' technologies.
Sorting biomolecules.	Nanopores and membranes are capable of sorting, for example, left- and right-handed versions of molecules.	Gene analysis and sequencing.	Current - ?

Figure 3 – Current and future uses of Nanotechnology  
<http://www.azonano.com/Details.asp?ArticleID=1078>

Within this paper, the technique of increased particle adhesion and the use of the material ligand on a nanoparticle surface will be explored in further detail. The focus will be on the immunotherapeutic treatment of Alzheimer's disease.

### Discussion

Alzheimer's disease is the most common disease that causes dementia, affecting around 465,000 people in the UK, and an estimated 16 million people worldwide, with that number likely to rise with the ageing population (Keating, 2005). It is a neurodegenerative dementia which is characterised by memory loss and cognitive impairment (Keating, 2005). Neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntingdon's diseases are often referred to as prion diseases and are associated with cerebral accumulation of abnormal protein deposits. In many cases (but not all) these deposits are largely composed of amyloid fibrils- highly ordered protein aggregates that show a characteristic 'cross- $\beta$ ' x-ray diffraction pattern (Dobson, 2003 cited in Ganchev et al, 2008). Amyloid precursor protein (APP) is a protein found throughout the body. The amyloid hypothesis states that a fault with the processing of amyloid precursor protein (APP) in the brain leads to the production of a fragment of APP known as beta-amyloid. Amyloid- $\beta$  peptide ( $A\beta$ ) is cleaved sequentially, first by  $\beta$ -secretase (forming  $APP\beta$  and the  $\beta$ -stub) and then by  $\gamma$ -secretase.  $A\beta$

is then thought to self-associate under certain circumstances to form amyloid plaques (see figure 4) (Schenk, 2002). The theory rests on the idea that it is the accumulation of this sticky protein fragment in the brain that triggers the disruption and destruction of nerve cells that causes Alzheimer's disease. The hypothesis is that there is a fault that either causes the over-production of beta amyloid or which interferes with the mechanism that usually clears it from the brain, or possibly both (Khan, <http://www.alzheimers.org.uk/site/scripts/documentsinfo.php?documentID=383&pageNumber=6>).

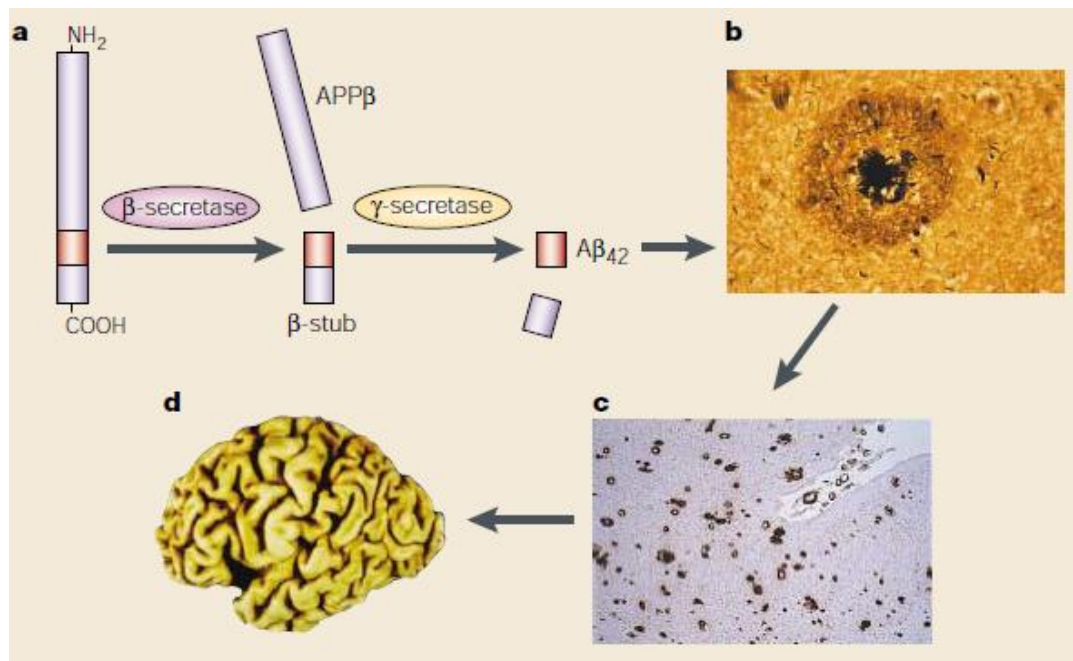


Figure 4-The production of  $\beta$ -Amyloid and formation of Amyloid plaques (Schenk, 2002, p.825, Box 1, <http://homepage.mac.com/sanagnos/schenk2002.pdf>)

There is no definitive diagnosis of the disease until after death, when the brain tissue can be examined for senile plaques and neurofibrillary tangles characteristic of the disease (Andreasen and Biennow, 2004 cited in Keating, 2005). Pre-mortem diagnosis, based on the patient's clinical history, in vivo brain imaging and neuropsychological, cognitive and neurological tests, is only 85% accurate (Knopman et al, 2001 cited in Georganopoulou et al, 2005). There are two general approaches to identifying soluble markers for AD. The first is based on measuring the total tau-protein or amyloid- $\beta$  protein concentration in cerebrospinal fluid (CSF) or plasma (Clark, 2003 cited in Georganopoulou et al, 2005). This approach lacks accuracy due to the significant overlap between marker levels in healthy and unhealthy subjects which has led to inconclusive results (Sunderland et al, 2003 cited in Georganopoulou et al, 2005). The second approach targets the suspected pathogenic markers, such as cleaved tau protein, phosphorylated tau protein, or amyloid- $\beta$  derived diffusible ligands (ADDL). Although this approach may lead to more definitive diagnosis, in the early stages of the disease, the concentration of such markers in CSF are so low that they cannot be identified accurately using the conventional methods of ELISA or blotting

assays (Georganopoulou et al, 2005). In response to this, a study by Georganopoulou et al, (2005) used the ultrasensitive bio-barcode assay to measure the concentration of amyloid –  $\beta$ -derived diffusible ligands (ADDLs), a potential soluble pathogenic AD marker, in the cerebrospinal fluid (CSF) of 30 individuals (15 with AD and 15 controls). A bio-barcode assay involves the homogenous isolation of certain antigens using gold nanoparticles and magnetised microparticles, both attached to antibodies specific to the antigen of interest (Georganopoulou et al, 2005). The ADDL concentrations for the subjects diagnosed with AD were consistently higher than the levels in the cerebrospinal fluid (CSF) taken from nondemented age-matched controls. This study is considered valuable for three main reasons. First, it shows that the highly sensitive bio-barcode assay can be used to measure the concentration of pathogenic ADDL in CSF at clinically relevant concentrations. This provides the opportunity to study a wide range of potential pathogenic markers in CSF which could lead to the development of more informative diagnostic tools for the disease (Georganopoulou et al, 2005). Secondly, it identifies that soluble pools of ADDLs that exist in the brain extend to the CSF and that higher levels of ADDLs correlate with the presence of the disease (Georganopoulou et al, 2005). Thirdly, the approach of using pathogenic markers in CSF combined with the barcode assay highlights the potential to develop a detection tool that is more reliable, faster and less expensive than current diagnostic methods (Georganopoulou et al, 2005). Before this assay can be used clinically a much larger study is required. The extraordinary sensitivity of this tool highlights the potential of developing a diagnostic tool, based on soluble pathogenic markers for the debilitating disease (Georganopoulou et al, 2005). Ultimately, this could lead to identification of ways to treat the disease.

Findings from Georganopoulou et al (2005) highlight the potential for the use of nanotechnology to provide a more effective form of identification of early-onset Alzheimer's. In the future, there is also the possibility that nanotechnology can help not only with early diagnosis but also the treatment of Alzheimer's disease, for example, the use of nanotechnology to specifically target beta-Amyloid proteins within the brain. Both the identification and treatment would be significant clinical advances in the fight against Alzheimer's disease. Whilst not using nanotechnology, the findings of Schenk et al (1999 cited in Schenk, 2002) highlight the possibility of targeting  $\beta$ -amyloid proteins via immunotherapy. Schenk et al (1999 cited in Schenk, 2002) conducted a trial on transgenic mouse models of the disease and received positive results, showing that immunization against  $\beta$ -Amyloid could provide protection and often reversal of the pathology of Alzheimer's disease in animal models. Since this research, many other clinical trials and experiments on mice have been conducted and results were promising for the development of a working immunotherapy for Alzheimer's. However, the most recent human trial returned disappointing results, with a small but nevertheless significant number of candidates returning symptoms of meningoencephalitis. This result shall be discussed in more detail in time. There are currently three  $\beta$ -amyloid immunotherapy strategies; the first is direct immunisation with a synthetic form of  $\beta$ -amyloid<sub>42</sub>, which stimulates T-cell, B-cell

and microglial immune responses; the second is also a form of active immunisation, using fragments of synthetic  $\beta$ -Amyloid combined with a carrier protein providing the necessary helper T-cell epitopes to stimulate B-cells to produce antibodies. The third strategy undergoing investigation is the passive administration of monoclonal antibodies against  $\beta$ -Amyloid, negating the need for an immune response to be made by the patient against the  $\beta$ -Amyloid protein, yet still being sufficiently strong in its defence of the body as to have protective effects and even reverse some of the symptoms of the disease.

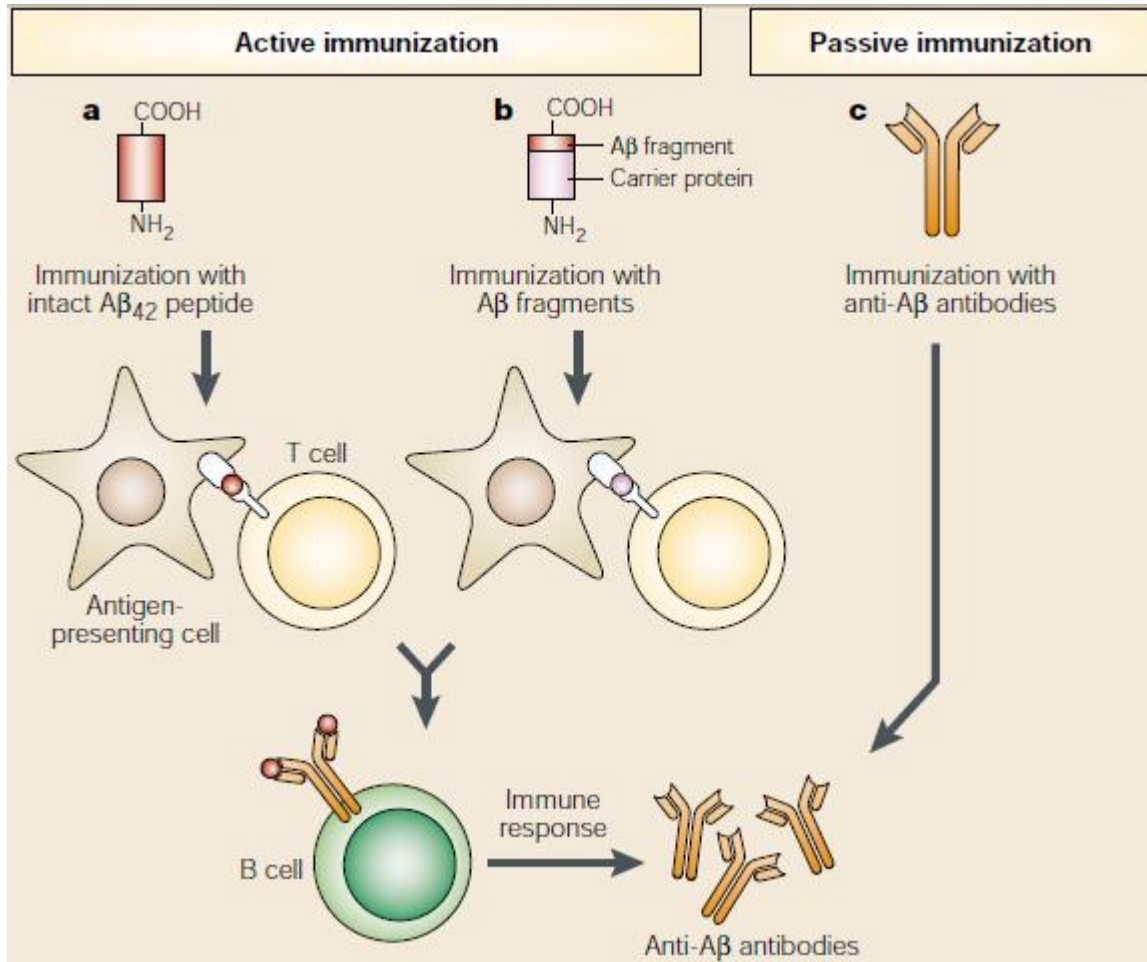


Figure 5 – Strategies for  $\beta$ -Amyloid immunotherapy

(Schenk, 2002, p.826, Box 2, <http://homepage.mac.com/sanagnos/schenk2002.pdf>)

In 1999, an active  $\beta$ -Amyloid immunotherapy drug known as AN1792 (Elan Pharmaceuticals, Ireland) was placed into early clinical trials after extensive pre-clinical studies, and its Phase I study appeared to confirm its safety for use, despite the original fears of some scientists about stimulating an immune reaction within the brain. The Elan/Wyeth team conducting these trials therefore continued with their testing and began Phase IIa trials in October 2001. They enrolled 372 patients with mild to moderate Alzheimer's disease, randomizing them and injecting either the AN1792 drug or placebo in

the ratio 4:1 in a series of doses at the beginning of the study and months 1, 3, 6, 9 and 12. However, the trial was terminated in January 2002, after symptoms of brain inflammation consistent with aseptic meningoencephalitis were reported in 6% of the patients (18 of 298) injected with the AN1792 drug (Orgogozo et al, 2003). Despite this clearly adverse effect of the treatment, many of the participants demonstrated evidence of the clearance of amyloid plaques, suggesting that the treatment in itself is still feasible for use, though significant changes in its application shall have to be made to avoid the health risks presented by this study (Town, 2009).

It was with this predominantly successful principle of treatment in mind that another study was carried out, this time using a passive form of anti- $\beta$ -Amyloid immunotherapy. As mentioned previously in this paper, there are three current ways of delivering the immunotherapy treatment, one of which was by injecting the patient with monoclonal anti- $\beta$ -Amyloid antibodies designed to be specifically complimentary to the antigens on the surface of the  $\beta$ -Amyloid protein. Theoretically, this approach should be successful in both removing Amyloid plaques and 'circumvent[ing] the potentially unsafe and damaging autoaggressive CD4+ T cell response that was presumed to have mediated aseptic meningoencephalitis in a small percentage of AN-1792 recipients' (Town, 2009, No page number). The Elan/Wyeth team continued in their trials, using the monoclonal antibody AAB001 (Bapineuzumab) in a multiple ascending dose clinical study, once again using patients with mild to moderate Alzheimer's disease. The 234 participants in the study were randomly selected for doses of either AAB001 or placebo in four groups of increasing dose strength (0.15, 0.5, 1.0 or 2.0 mg/kg). They received 6 separate doses, each 13 weeks apart. On the 78<sup>th</sup> week, final assessment and analysis occurred and findings were disappointing as 'due to varying doses and a lack of statistical precision, this Class II ascending dose trial provides insufficient evidence to support or refute a benefit of bapineuzumab' (Salloway et al, 2009, Conclusions, No page number). In addition, there were cases of vasogenic edema (Figure 6), a form of cerebral edema that forms when fluid builds up in the intra/extracellular areas of the brain, in 9.7% (12/124) of patients. Although this condition did subside and reverse eventually, there are clearly still significant health risks with current methods of treatment and the strategies associated with its delivery.

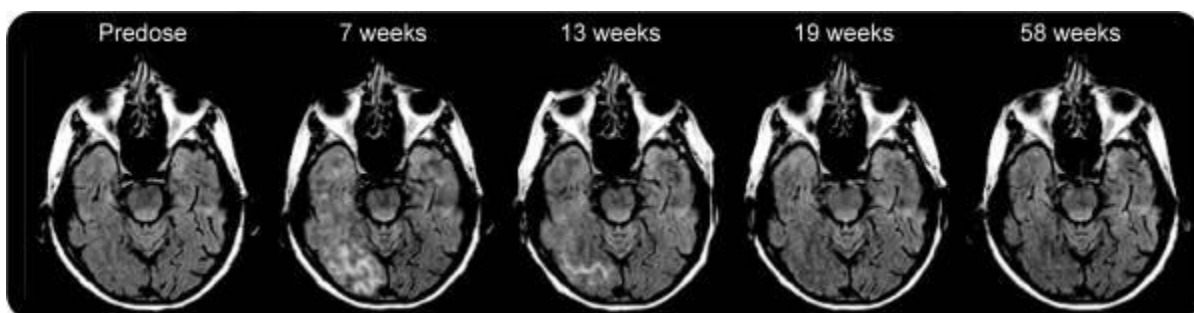


Figure 6 – Magnetic Resonance Imaging (MRI) scans of an AAB001 patient with vasogenic edema (Salloway et al, 2009, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2790221/figure/f3-7201/>)

It is the aforementioned weakness of the treatment program that leads to our main hypothesis. The cerebral inflammation that occurred in both the above clinical studies has been attributed to the use of a different adjuvant (a substance injected alongside a drug or antigen to enhance their medical effectiveness and the immune response stimulated by the antigen), pro-inflammatory Th1 adjuvant QS-21 rather than anti-inflammatory Th2 Complete Freund's adjuvant which is prohibited for use in humans (Town 2009). 'If this were the case, then strategies that introduced the A $\beta$  immunogen with alternative adjuvants (perhaps anti-inflammatory Th2 adjuvants) or no adjuvant and/or relied on a different route of administration (*i.e.*, not intraperitoneal, intramuscular, or intravenous) may be both efficacious and safe' (Town, 2009, No page number). Perhaps the world of nanotechnology could be used in combination with existing research into treatments for diseases, in this case Alzheimer's, to find a solution that would benefit mankind. To be more specific, one could use such nanotechnology as 'Ligands on a nanoparticle surface' to recognise the  $\beta$ -Amyloid plaques and attach to them, as 'the multivalent conjugation of targeting ligands on the surface of nanoparticles is presumed to enhance binding to the desired target' (Tassa et al, 2010, Abstract). They would then release a form of the immunotherapy, be it active or passive, resulting in very localised immune responses and therefore less swelling – avoiding the health problems that had previously plagued this form of treatment. The nanoparticles themselves would theoretically help keep swelling localised, as they increase surface adhesion of particles which thereby increases localised drug retention, ensuring that the immunotherapy only occurs in the sites containing  $\beta$ -Amyloid plaques. In addition to keeping the immune response restricted to a specific area to reduce overall brain inflammation, one could also attach an anti-inflammatory drug or some substance evoking an anti-inflammatory immune response (such as a Th2 adjuvant) to the ligand nanoparticle, thereby decreasing swelling in the brain.

Whilst nanotechnology holds tremendous potential for medical innovation it should be acknowledged that there may be potential risks and hazards associated with it. There is some evidence that certain nanoscale particles can have detrimental effects on living organisms (McNeil, 2005). Whether actual or perceived, the potential health risks associated with the manufacture, distribution and use of nanoparticles must be balanced by the overall benefits that such technology has to offer in both therapeutic and diagnostic applications (McNeil, 2005). The diversity of nanotechnologies is considered likely to prevent a one-solution-fits-all approach to risk (Maynard, 2007). To address the issue of risk, it is important that the nanotechnologies presenting a clear threat to health must be distinguished from those less likely to cause harm. Maynard and Kuempel (cited in Maynard, 2007,p.7) propose two criteria for identifying nanomaterials which may present a unique potential risk to human health:

- (1) 'The material must be able to interact with the body in such a way that its nanostructure is biologically available (i.e. exposure must occur, and the material's nanostructure must be biologically accessible following exposure).'
- (2) The material should have the potential to elicit a biological response that is associated with its nanostructure (i.e. the potential should exist for a response that differs from that associated to a non-nanoscale material of the same composition).'

There is evidence that we have been exposed to certain types of nanoparticles in the atmosphere for years without any risks to health. However, there is also evidence that exposure to nanoparticulate matter from sources such as vehicle exhausts and welding fumes is associated with increased risks to health (Whatmore, 2006). There is also evidence that, should nanoparticles lodge in the lungs, they may cross the barrier into the blood stream and possibly migrate to other parts of the body (Whatmore, 2006). Further research is required as a matter of urgency to identify the possible risks to health and for informed decisions to be made about the future use of nanotechnology.

### Conclusion

To conclude, nanotechnology holds tremendous promise for answering some of biology's most challenging biochemical and genetic questions (McNeil, 2005). Within this paper the potential for nanotechnology to be used in the detection and treatment of Alzheimer's disease has been explored. Alzheimer's is a debilitating disease which currently cannot be accurately diagnosed before post-mortem. At this time, there are no effective treatments available for the disease. As the life expectancy of the population grows, the number of cases of Alzheimer's is likely to increase. The need to effectively diagnose and treat this disease is of utmost and increasing importance. Existing studies have highlighted the potential for nanotechnology to be used in the identification of the disease (Georganopoulou et al, 2005). The targeting of  $\beta$ -Amyloid proteins has been identified as a potential way to treat the disease (Schenk et al, 1999). By building on this existing medical knowledge, further advances can be made by developing nanotechnology which specifically targets  $\beta$ -Amyloid plaques within the brain. The potential risks both during research, manufacture and during clinical trials cannot be underestimated. Further research is therefore required on two key fronts. The key hypothesis of this paper depends on theoretically sound ideas being applied within the practical world. For this to happen, further research is required on the use of nanotechnology. As Maynard (2007, p.4) acknowledges '...Little is still known about what the immediate risks may be, or how to handle them. Still less is known about how we might predict and manage risks from new technologies in the coming years'.

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