A REPORT RESEARCHING THE APPLICATION OF NANOTECHNOLOGY IN THE DETECTION AND TREATMENT OF CANCER

BY
CHARLOTTE ALLAN

PASS WITH MERIT

RESEARCH PAPER
BASED ON
PATHOLOGY LECTURES
AT MEDLINK 2010

Abstract

The subject of this paper is the research and development into nanotechnology, building machines from individual atoms. I will be focusing on the research and the future use of nanotechnology in the diagnosis, treatment and prevention of cancer. In the near future it is very possible that devices could be manufactured that have the ability to detect malignant cells, image the cells, either remove or repair them and even prevent cells from becoming cancerous. There are many different applications of nanotechnology that I have discussed and they could completely impact on current clinical methods used in the treatment of cancer, also removing many of the distressing side effects associated with them. Although there are several issues about nanotechnology that require consideration it appears the future for oncology will be transformed.

Introduction

Nanotechnology is the engineering of manipulating matter at a subatomic level. It includes creating machines and robots with highly complex functions that are only a few nanometres in size, just tiny fractions of the width of a human hair. When working at the nanoscale the physical, biological and chemical properties of a material significantly differ from those of the corresponding material in bulk. By creating nanoscale structures it is possible to control the properties of a material including its melting point and sometimes even its colour without altering its chemical composition. The term 'Nano' is the Greek word for 'midget'. Nanotechnology was first introduced in 1959, when the American Physicist, Richard Feynman made a speech entitled 'There's plenty of room at the bottom' in which he considered the possible manipulation of individual atoms as the latest discovery in synthetic chemistry.

In 1986, Eric Drexler published 'Engines of Creation' which discussed the possible future for nanotechnology in medicine. One possible application which Drexler discussed was cell repair machines in which machines comparable to the size of bacteria will travel through tissue and into cells – in a similar way to viruses, and once inside the cell will examine its contents and activity and then take action where required. It is believed that early machines will be highly specialised and detect and repair one type of molecular disorder such as deficiencies. However, later forms of cell repair machines could be programmed to deal with more general issues. Nanocomputers will be required to guide more complex machines. Their job will be to control simple computers which in turn will direct the cell repair machines to those cells which need examining and repairing. In this way, working molecule by molecule these machines will have the ability to repair entire cells. With the aid of larger devices they could repair entire organs and with their ability to synthesize complete molecules and cells they could replace those damaged beyond repair. As cells naturally contain chemicals that can power nanomachinery there is no problem with powering the devices. The use of cell repair machines could bring a significant breakthrough; freeing medicine from a reliance on self-repair.

The next breakthrough in nanotechnology was during 1985 when the first fullerene was discovered by Kroto, Curl and Smalley. A fullerene is a molecule composed entirely of carbon in the form of a sphere, tube or ellipsoid. Spherical fullerenes were also called buckyballs because of their similarities to a football (Figure 1) and the cylindrical fullerenes are called carbon nanotubes

(Figure 2) and they both have a very similar structure to graphite. These fullerenes could be used to help create nanomachines because of their suitable chemical and physical properties – they are exceptionally strong but weigh very little. The strength and flexibility of the carbon nanotubes makes them possibly suitable to control other nanoscale structures, which suggests they could have a vital role in the engineering of nantechnology. Because of the carbon nanotubes' superior mechanical properties many applications have been researched ranging from everyday items to space elevators. Work initiated by Ray. H. Baughman revealed that single and multi-walled nanotubes can synthesize structures with toughness unmatched in both the natural and man-made worlds. One area of research instigated by this included weaving these materials into clothing creating stab and bullet proof vests as they could prevent them from penetrating the body.

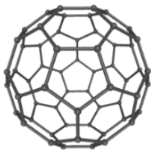

Figure 1 – A buckyball

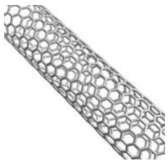

Figure 2 – A carbon nanotube

Several applications for nanotechnology are currently being used; sunscreens and cosmetics to name a few. However it is anticipated that in the coming decades new phases of products will be introduced, such as complex electronic products, which will have vast implications.

<u>Discussion</u>

The focus of my research is the use of nanotechnology in detecting, diagnosing and treating various forms of cancer.

With this successful research into nanomachinery that could travel in the body and detect and repair damage to cells, the use of nanotechnology in the detection and treatment of cancer was hugely anticipated. Currently the National Cancer Institute (NCI) in America are engaged in efforts to use the power of nanotechnology to totally eradicate death and suffering from cancer by 2015. Cancer is caused by the alteration of the genetic code in cells, resulting in changed expression of gene products, RNA and proteins. Currently cancer is being missed at the earliest stages, which is partly due to detection methods not being directed at cellular changes of carcinogenesis. 'Nanotechnology will help define cancers by molecular signatures denoting processes that reflect fundamental changes in cells that can lead to cancer.'

New technologies will be required to create tools that have the ability to examine samples which, in turn, will create an entirely new system of tackling cancer integrating detection, diagnosis and intervention. 'Nanodevices will provide noninvasive access to living cells which offers the ability to allow simultaneous interaction with several critical proteins and nucleic acids at the molecular scale which should provide us with a clearer understanding of the complex signalling networks that govern the behaviour of cells in their normal state and as they undergo malignant transformation.' Nanoscale cantilevers, built as part of a larger device, can offer swift and accurate detection of cancer-related molecules.

Research supported by the NCI into nanodevices developed to address cancers has shown they have the capabilities to 'detect cancer at its earliest stages, pinpointing its location within the body, delivering anticancer drugs specifically to malignant cells and determining whether these drugs are killing the malignant cells.' The outcome of this research is very positive and there is great certainty that nanotechnology will completely transform the very foundations of cancer treatment worldwide.

Researchers are currently developing an array of nanoscale particles to act as diagnostic platform devices. One example is when DNA-labelled magnetic nanobeads which have the potential to be used as a changeable foundation detecting almost any protein or nucleic acid with much greater sensitivity than the current more conventional methods. If this proves true for a large quantity of these systems, nanoscale diagnostics could offer the means of converting even the rarest biomarkers into constructive diagnostic indicators.

Another area of cancer assessment which could be hugely enhanced by the application of nanotechnology is the delivery of anticancer drugs. Nanoscale structures have the potential to significantly improve cancer therapy and to hugely increase the amount of effective therapeutic agents. Chemotherapeutic agents or therapeutic genes could be transported in doses by nanoscale constructions, which act as customizable target drug delivery vehicles, into malignant cells whilst sparing healthy cells which should significantly reduce the distressing side effects associated with many current cancer treatments.

'Already, research has indicated that nanoscale drug delivery systems such as dendrimers (spherical, branched polymers), silica coated micelles, ceramic nanoparticles and cross-linked liposomes can be targeted to cancer cells.' This is achieved by connecting monoclonal antibodies or cell-surface receptor ligands that bind exclusively to molecules situated on the surfaces of cancer cells, such as the luteinizing hormone releasing hormone (LH-RH), or 'molecules unique to endothelial cells that become co-opted to malignant cells.' (Figure 3) Once they reach their target, the nanosized particles are swiftly taken up by the cells. As further research has revealed other molecules which are exclusive to cancer cells, targeted nanoparticles could become the most effective method of delivering anticancer drugs directly to tumour cells and their adopting endothelial cells.

Ultimately this could lead to the possibility to mix and match the drugs with any one of a range of these nanoscale delivery systems and targeting agents. This would give scientists the opportunity to perfect therapeutic properties without the use of new bioactive molecules.

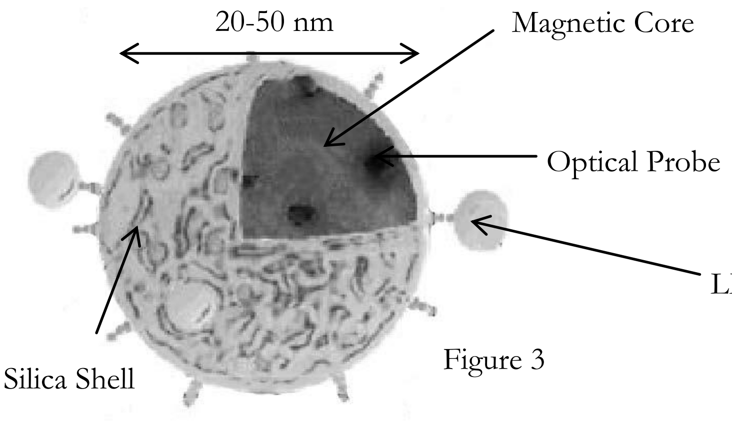

Figure 3

Figure 3: Multifunctional nanoparticles can be targeted to cancer cells using receptor ligands.

The current research into the use of naturally - existing nanodevices appears to be very positive and holds great promise for the future, but engineers and chemists have already made significant breakthroughs turning synthetic materials into multiuse nanodevices. Dendrimers, 1-10 spherical, branched polymers, are proving particularly skilful at providing multifunctional modularity. In one demonstration chemists attached folate which targets the high-affinity folate receptor located on certain malignant cells, the indicator fluorescein, and either of the anticancer drugs methotrexate or paclitaxel to a single dendrimer. (Figure 4) Experiments, both *in vivo* and *in vitro,* have shown that the therapeutic drugs were delivered successfully to the folate receptor-positive cells by the nanodevices whilst simultaneously recognising these cells for fluorescent detection. Further work, in which a fluorescent indicator was linked to the dendrimer, proved that not only were the devices delivering the drugs to the appropriate cells but that they were also having the desired effect. Some devices very similar to these have been involved in clinical trials for treating a variety of cancers.

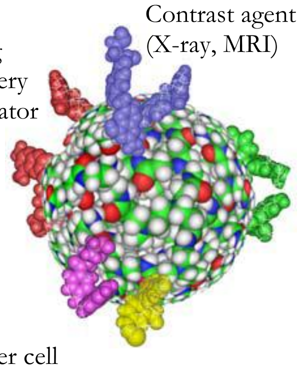

Figure 4

Figure 4: Dendrimers can serve as versatile nanoscale platforms for creating multifunctional devices capable of detecting cancer and delivery drugs.

Therapeutic

Another tool which has also been used within the targeted nanodevices is the photosensitizer, which is used in photodynamic therapy. Light is used to generate reactive oxygen locally within tumours. The next stage in this research is to entrap a light-generating system aswell in such a way as to activate light production only once the nanoparticles are taken up by a targeted cell. If this research proves successful, such an approach would hugely extend the practicality of photodynamic therapy to include the treatment of tumours deep within the body.

Such interchangeable nanodevices hold the potential of fundamentally changing the practice of oncology, possibly with the ability to detect the first indications of cancer and provide effective therapeutics throughout the earliest stages of the disease. It is certainly envisaged that one day a nanodevice will be able to fingerprint a certain cancer and dispense the correct drug at the suitable phase in a malignant cell's life cycle, making customised medicine a reality at the cellular level.

'An important aspect of biomedical nanotechnology research is that most systems are being designed as general platforms that can be used to create a diverse set of multifunctional diagnostic and therapeutic devices.'

One clinical trial, led by S. K. Libutti, researched the safety and efficacy of systemically administered tumour necrosis factor alpha (TNF). TNF is attached to a nanoparticle (PEGylated colloidal gold particles) by a covalent bond. Each component of CYT-6091 serves a specific function in achieving tumour-targeted drug delivery and ensuring a successful outcome. The patients included in the trial had to meet the following criteria:

- Advanced and/or metastatic histologically documented solid organ cancer
- Tumour that is refractory/untreatable with conventional drugs.
- Measurable or evaluable metastatic disease
- ECOG performance of <2 and expected survival of more than 3 months
- No treatment with cytotoxic or biologic treatments 3 weeks prior to dosing.

The aims of the trial were to determine a maximally tolerate dose (MTD) and characterise the dose limiting toxicities (DLTs) of CYT-6091 administered intravenously (IV). Other secondary aims of the trial were to establish: the pharmacokinetics of CYT-6091 following IV administration, the presence of CYT-6091 in the tumour and in healthy tissue, whether there is an antibody response to CYT-6091 and if anti-tumour effects occur.

The pre-clinical trial results were as follows:

- Well tolerated TNF formulation
- No uptake by the liver or spleen
- Gold nanoparticles present in the tumour
- 10x more TNF detected in the tumour
- Effective anti-tumour response in mice
- Clinical response recorded in terminally ill dogs and cats

The trial established the following about CYT-6091:

- Exceeds the historic MTD of TNF without DLTs
- Induces a predictable and controllable febrile response
- Does not induce hypotension
- No effect on renal, liver or immune function
- Does not induce an anti-TNF antibody response
- Well- tolerated, no unexpected adverse events reported
- Increases the circulatory half-life of TNF
- Is primarily detected in tumours, but not in healthy tissue

These results appear to be very positive and they show that the nanodevice is very efficient at directing the treatment at the tumour whilst avoiding healthy tissue. One adverse effect which was linked to the treatment were low lymphocyte counts in the blood. However they were not sufficiently low to warrant been reported as an 'adverse event'. Although this may appear insignificant in retrospect to the tumour it does leave the patient at a greater risk of infection so should be taken into consideration. This investigation helps outline how effective these delivery systems appear to be.

Intravascularly injectable nanovectors are a significant group of nanotechnological devices of interest for use in cancer. It is thought they will be used for the in vivo, non-invasive visualisation of markers of first stages of disease; the directed delivery of therapeutic agents with a simultaneous, considerable reduction of harmful side effects; and – by an amalgamation of these – the interception and confinement of lesions before they reach the malignant phenotype, with very marginal concurrent reduction in quality of life. The simplest form of the nanovectors are liposomes. 'They use the overexpression of fenestrations in cancer neovasculature to increase drug concentration at tumour sites. Liposome-encapsulated formulations of doxorubicin were approved 10 years ago for the treatment of Kaposi's sarcoma, and are now used against breast cancer and refractory ovarian cancer.' The liposomes were then refined and adapted to more cancer indications. These liposomes are the first in a growing number of nanovectors currently being researched for more efficient drug delivery systems.

Current medical cancer imaging technologies are unable to detect based on lesion anatomy because of insufficient spatial resolution. Contrast agents are required to identify malignancies based solely on their molecular expression profiles by means of imaging. These contrast agents are composed of a material that amplifies signals conjugated to a molecular recognition and targeting agent for example an antibody. Currently scientists are developing multifunctional, molecularly or physically targeted contrast agents for all imaging modalities, with the intention to detect both smaller and early-stage tumours, providing an improved structural clarity for lesions and detecting molecular expressions of neoplasms. This was demonstrated by Weissleder and colleagues when they showed that 'lymphotropic paramagnetic nanoparticles allow the MRI imaging of clinically occult lymph-node metastases in patients suffering from prostate cancer', which were unidentifiable by all other non-invasive approaches. The lymphatic drainage of breast cancer in mice was imaged by polymeric dendrimers which were used as gadolinium nanocarriers, illustrating that this strategy could be used as a replacement for sentinel lymph-node biopsy. 'Dextran-coated, ultra-small paramagnetic iron-oxide nanoparticles were shown to outperform conventional gadolinium MRI contrast in terms of intraoperative permanence of imaging enhancement, inflammatory targeting, and detectability at low magnet strength in the surgical treatment of brain tumours.'

One key marker in the early detection of cancer which is found in pre-malignant lesions of the cervix, breast and skin, is continuous angiogenesis which is usually anticipated as an early – to – midstage occurrence in human cancer. In various research groups nanoparticles have been used to successfully image angiogenesis with MRI in animal models. The signals emitted from very low picomolar concentrations of epitopes targeted by appropriate nanoparticles were detected by MRI which shows great potential for clinical processes in the future.

Researchers have also been identifying where they can refine technology platforms for the detection of cancer biomarkers *ex vivo*. One thing that is unavailable for the early detection of most cancers are serum markers. The markers that are currently in use, for example prostate-specific antigen (PSA) and carcinoembryonic antigen (CEA), are all non-specific and have hugely varied baseline expressions in the population, so are of limited effectiveness for early detection. Remaining of paramount importance is the aim to develop dependable methods for early detection from serum, other biological fluids, or any sample obtained from minimally invasive procedures. Beginning with surface patterning approaches there are numerous nanotechnologies that are practical candidates for early detection platforms involving firmly established technologies such as DNA microarrays, and SELDI-TOFF mass spectroscopy for proteomics. 'For these approaches, the transition from the micron to the nanoscale dimensional control on surface features translates into increases in information quality, quantity and density.'

The approaches that could facilitate the conversion from single-biomarker to multiple biomarker cancer diagnostics, prognostics and treatment selection could be nanocantilever, nanowire and nanotube arrays, but they do have limitations. One area for concern in these approaches is the requirement for covalent bonding between distinct antibodies or other biological recognition molecules to the devices. One key obstacle to overcome to allow the analysis of proteomic signatures will be the recognition of signatures from low – concentration molecular species when extremely high concentrations of non-specific serum proteins are present.

There is also potential for nanoparticles to be involved in the *ex vivo* detection of biomarkers. For example, fluorophore – laden silica beads can optically identify leukaemia cells in blood samples; gold – nanoshell – based immunoassays have been established; fluorescent nanoparticles have been used in an extremely sensitive DNA – detection system; and quantum dot bioconjugates with targeting antibodies can identify molecular signatures. Stability and 'tunability' are among the advantages that nanoparticles have against conventional staining methods, one example is that quantum dots signal intensity always remains constant; they do not 'photobleach'. Furthermore, communities of nanoparticles, each with a distinct colour, might be conjugated with antibodies to different molecular targets. Once irradiated an exact map of the placement of multiple molecular markers in a single cell or tissue is created. Many possible benefits are created by this for example 'readily identifying the conjugate markers; yielding specific information on their tissue distribution and introducing new protocols that include cell surface, endocellular and microenvironmental antigens in the same test.'

One application of nanoparticles as particular, enriching harvesting agents for serum proteomics has been suggested. The emphasis for this method is on low – molecular – weight proteolytic fragments, which are detected in trace quantities in several cancers particularly ovarian.

Conclusion

Although nanotechnology is thought to be cost effective as the cost to manufacture the materials is only marginally bigger than the cost of the raw materials and energy, the cost of research into synthesising these nanodevices is massive; one project instigated by the NCI to research the use of nanotechnology in diagnosing, treating and preventing cancer had a budget of $4-6 million in 2010 alone. The cost however may seem insignificant as cancer is the leading cause of death worldwide; 7.9 million people died from cancer in 2007, but it could hinder progress in the pursuit to eliminate this debilitating disease.

The use of nanotechnology carries many risks particularly because of our lack of understanding of what effect these nanoparticles could have on our biological processes and systems as the idea was only first discovered in 1959 so is relatively 'new'. Great issues have also arisen about the disposal of nanowaste, there is conflicting data about how hazardous nanotubes and fullerenes are; some state they are highly flammable whereas others disagree. There is also concern over the disposal of nanomaterials that contain toxic elements such as arsenic or cadmium. It is thought that the only safe method of disposal for many nanomaterials will be incineration or landfill. There is also speculation over the safety of carbon nanoparticles in the body, it is believed they could increase blood clotting which could potentially block large arteries so further studies will be required to confirm/deny these suspicions.

Ethical issues associated with nanotechnology could arise as Dr. Raj Bawa explains "Interventions based on nanotechnologies will likely resurrect old questions about human enhancement, human dignity, and justice that have been asked many times before in the context of pharmaceutic research, stem cell research and gene therapy." The basis of nanotechnology questions our right to interfere with the natural processes within the human body and there is huge debate as to where the use of nanotechnology would stop; the construction of cells instead of reproduction? Synthesising meat instead of slaughtering animals? Some may believe that we should protect life at all costs; eradicating all life-threatening diseases, however many others feel this is unnatural and changing the life God has created for them. Another aspect which requires great consideration is how these nanodevices will be distributed to all areas of the world, especially the most deprived; due to their dependency on the technology in the modern world. This could help cure the simplest diseases worldwide, potentially saving thousands of lives. Scientists researching and developing nanodevices are aware they could be held liable if the products they have constructed cause significant harm to patients, therefore they need to ensure their products are safe for use only with foreseeable side effects. The subject of privacy is also very worrying; with the use of data collecting devices it will be very difficult to monitor this data and how it is used.

The research and information currently available about nanotechnology that could provide massive technological advances so should also be made available to the public to maximise the potential nanotechnology holds. I believe nanotechnology could wholly transform the future of oncology, despite a few setbacks that could impede progress, research projects are constantly progressing and I am convinced that in the foreseeable future many cancers could be curable.

The Project on Emerging Nanotechnologies
http://www.nanotechproject.org/inventories/medicine/

The Engines of Creation http://e-drexler.com/d/06/00/EOC/EOC_Chapter_7.html#section02of08

Fullerenes http://www.ch.ic.ac.uk/local/projects/unwin/Fullerenes.html

Figure 1 http://upload.wikimedia.org/wikipedia/commons/4/41/C60a.png

Figure 2 http://www.nanoid.co.uk/nanotube_filter.html

Potential Applications http://en.wikipedia.org/wiki/Carbon_nanotube#Potential_applications

Emerging Developments and Early Detection of Cancer
http://www.atp.nist.gov/clso/dma00187.pdf

Nanotechnology, cancer http://nano.cancer.gov/objects/pdfs/Cancer_brochure_091609-508.pdf

Figure 3 Page 13 http://nano.cancer.gov/objects/pdfs/Cancer_brochure_091609-508.pdf

Figure 4 Page 15 http://nano.cancer.gov/objects/pdfs/Cancer_brochure_091609-508.pdf

Cancer Nanotechnology, opportunities and challenges
http://web.mit.edu/kym/OldFiles/Public/paper/cancer_nanotechnology.pdf

Cancer Nanotechnology Platform Partnerships
http://nano.cancer.gov/objects/pdfs/CNPP_Grodzinski.pdf

Ethical aspects of nanotechnology in medicine
http://www.nanowerk.com/spotlight/spotid=3938.php

Disposal/Recycling of nanomaterials
http:// www.cohesion.rice.edu/.../Mustafa_Nanomaterials%20Workshop-Houston-Texas(FINAL).ppt

The ethics of nanotechnology http://www.actionbioscience.org/newfrontiers/chen.html

Ethics of nanotechnology http://www.nanotech-now.com/ethics-of-nanotechnology.htm

Big troubles may lurk in super tiny tech http://articles.sfgate.com/2005-10-31/news/17396870_1_foresight-nanotech-institute-nanotechnology-industry-nanomaterials/2