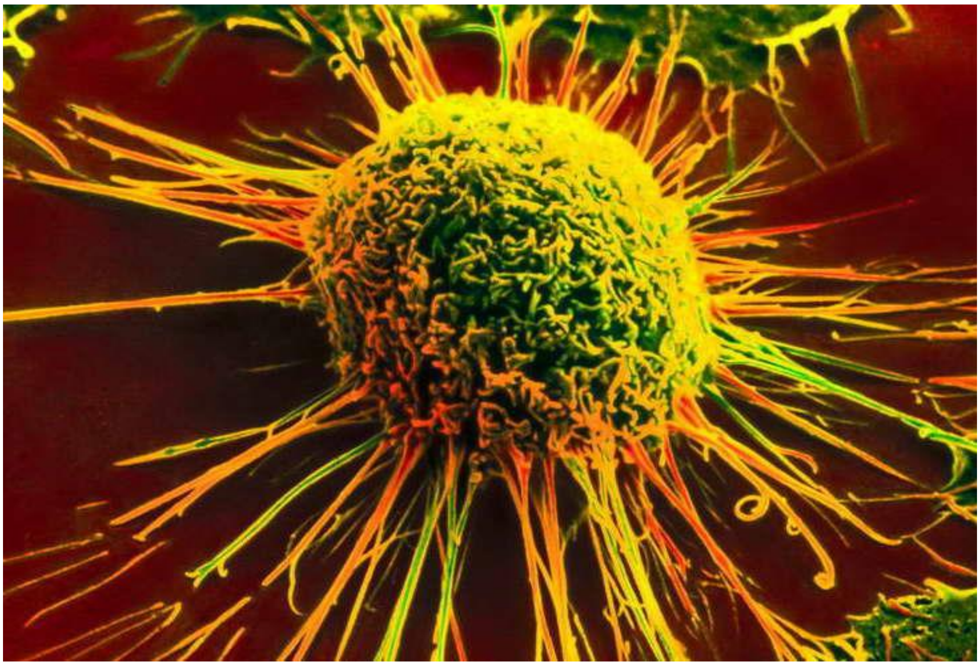
NANOTECHNOLOGY AND THE THERANOSTIC APPROACH FOR THE FUTURE MANAGEMENT OF CANCER AND THE ETHICS OF NANOMEDICINE



BY

CHIRAAG NITHIN HEGDEKAR

PASS WITH MERIT

RESEARCH PAPER BASED ON PATHOLOGY LECTURES AT MEDLINK 2010

Abstract

Nanotechnology is a multidisciplinary development involving engineering multifunctional devices with novel physical, chemical, and biological properties at the nano scale. These properties are due to the relative size of the device. Nanomedicine is application of nanotechnology to health. Molecular technology is destined to become the core technology underlying all of the 21st century medicine and dentistry. Nanomedicine has the potential to enable early detection, prevention, treatment and follow up of diseases. This paper discusses the application of nanotechnology in medicine such as imaging, targeted drug delivery, therapy, nanosurgery, tissue engineering and the ethical questions these techniques raise. In particular it focuses on the non-invasive theranostic (diagnosis and treatment) approach in cancer management.

Introduction

On December 29th 1959, late Nobel physicist Richard P Feynman gave a radical lecture titled “there is plenty of room at the bottom,” **(1)** and proposed that it should be possible to make machines at a nanoscale. This lecture was the birth of the idea and study of nanotechnology and what Feynman considered a possibility, is becoming a reality today.

Nanomedicine, refers to highly specific medical interventions at the molecular scale. The prefix nano comes from the Greek word *nanos*, or “dwarf” and means one-billionth (10^{-9}) of something: and the structures which are smaller than 100 nanometres (nm) in at least one dimension. Figure (1) shows a perspective of the nanoscale compared to other scales. Nanoparticles have a small size which avoid uptake by the Reticulo-endothelial system and large enough to avoid renal clearance and extravasation from blood vessels except at target tissue e.g., tumour cells where blood vessels are leaky.

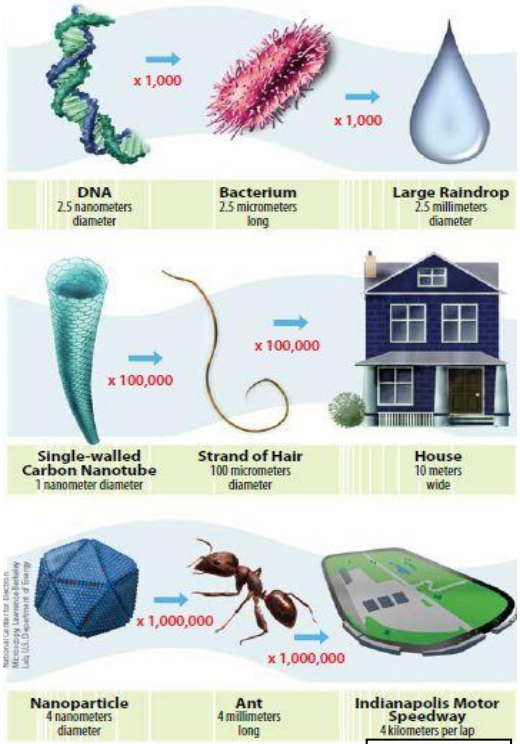


Figure 1

The design of nanoparticles is under active development and currently they can be classified as either first or second generation(2). Examples of first generation include the liposomes, albumin-bound NP etc. Second generation nanoparticles, due to novel molecular engineering, are able to target, image, deliver a therapeutic agent, and monitor therapeutic efficacy in real time.

The following are some of the examples of nanoparticles researched at present:

Fullerenes

Buckyballs and carbon tubes are both members of the fullerene structural class and are carbon-based. Buckyballs are named after American architect, Buckminster Fuller who designed it and won the Nobel Prize in 1996. It has molecules made up of 60 carbon atoms in a series of hexagons and pentagons, forming a structure similar to a football (fig 2). They have many astonishing properties and applications from drug delivery to energy transmission, yet all their toxicities are still unclear.

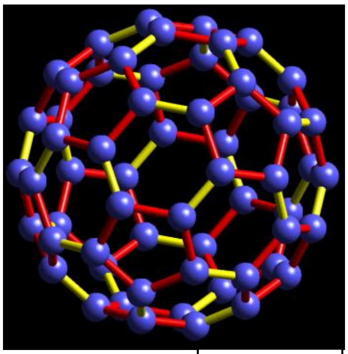
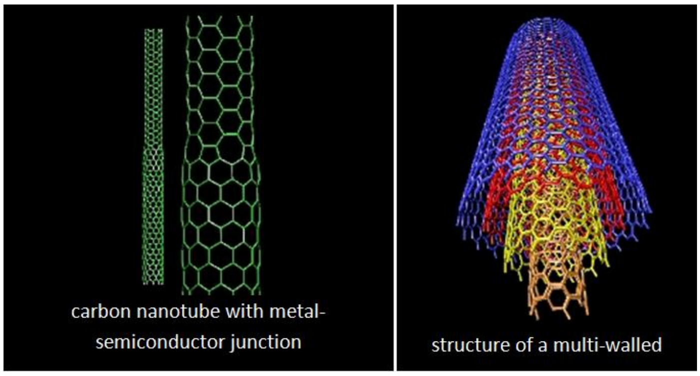


Figure 2

Nanotubes

Nanotubes are a sheet of graphite rolled into a cylinder(fig 3). Single-walled carbon nanotubes (SWCNTs) can be formed by rolling a single layer of graphite into a cylinder; while multi-wall carbon nanotube (MWCNT) can be a coaxial assembly of SWCNT cylinders. Its potential applications are in drug delivery and bio-sensing methods for disease treatment and health monitoring.



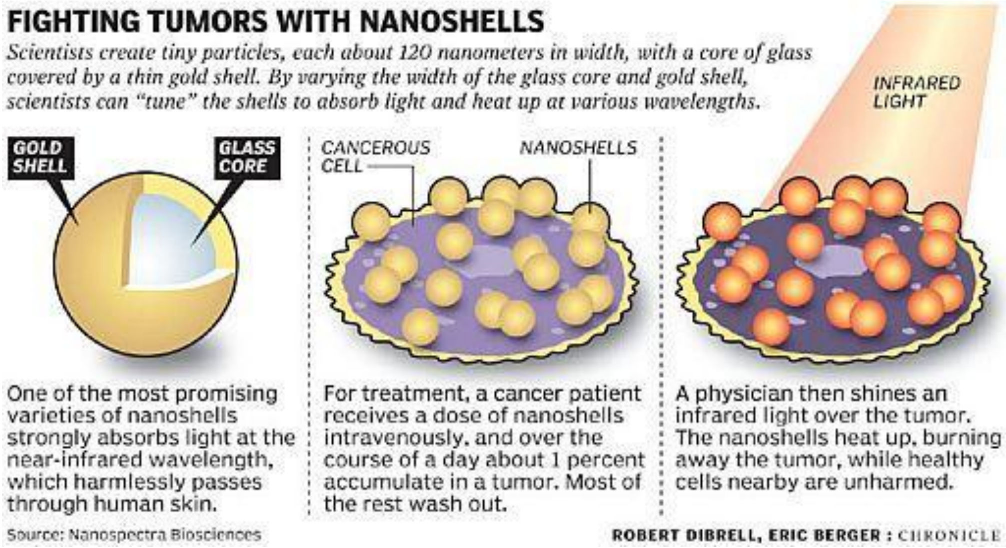
carbon nanotube with metal-semiconductor junction

structure of a multi-walled

Figure 3

Nanoshells

Nanoshells are spherical cores of a compound surrounded by an outer coating of another. When gold-coated particles are injected into the bloodstream they selectively accumulate in tumours. Nanoshells can be tailored to convert near-infrared light into heat by varying the size of the core and the thickness of the gold shell. A nanoshell has a large surface area exposed and the electrons on the surface of the metal are free to move around causing the nanoshell to heat up, which destroys tumour cells (fig4). The heating is localized and does not affect healthy tissue close to the tumour. Nanoshells can be used in tumours that are difficult to access like the head and neck.



FIGHTING TUMORS WITH NANOSHELLS

Scientists create tiny particles, each about 120 nanometers in width, with a core of glass covered by a thin gold shell. By varying the width of the glass core and gold shell, scientists can "tune" the shells to absorb light and heat up at various wavelengths.

One of the most promising varieties of nanoshells strongly absorbs light at the near-infrared wavelength, which harmlessly passes through human skin.

For treatment, a cancer patient receives a dose of nanoshells intravenously, and over the course of a day about 1 percent accumulate in a tumor. Most of the rest wash out.

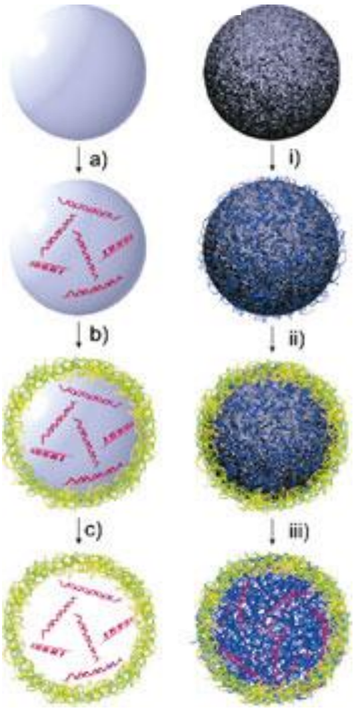
A physician then shines an infrared light over the tumor. The nanoshells heat up, burning away the tumor, while healthy cells nearby are unharmed.

Source: Nanospectra Biosciences

ROBERT DIBRELL, ERIC BERGER : CHRONICLE

Figure 4

Figure 5



Small interfering RNAs (siRNAs) can prevent certain proteins from being produced in a cell. Microcapsules can be used to deliver siRNAs as they have a special property to withstand the oxidising conditions of the bloodstream whereas siRNAs cannot on their own. The siRNa can stop the over-production of survivin (a cancer protein that prevents the cell from dying) thus causing the tumour cell to die at the normal time (fig5).

Magnetic nanoparticles

Magnetic nanoparticles are a class of nanoparticles which can be manipulated using magnetic field. These particles commonly consist of a core of magnetic elements and a biocompatible surface coating that provides stability under physiological conditions (fig6). The latter can be chemically modified to allow attachment of biomolecules. Superparamagnetic iron oxide nanoparticles (SPION)$^{(3)}$ are applied for drug delivery and gene transfection (process of deliberately introducing nucleic acids into cells).

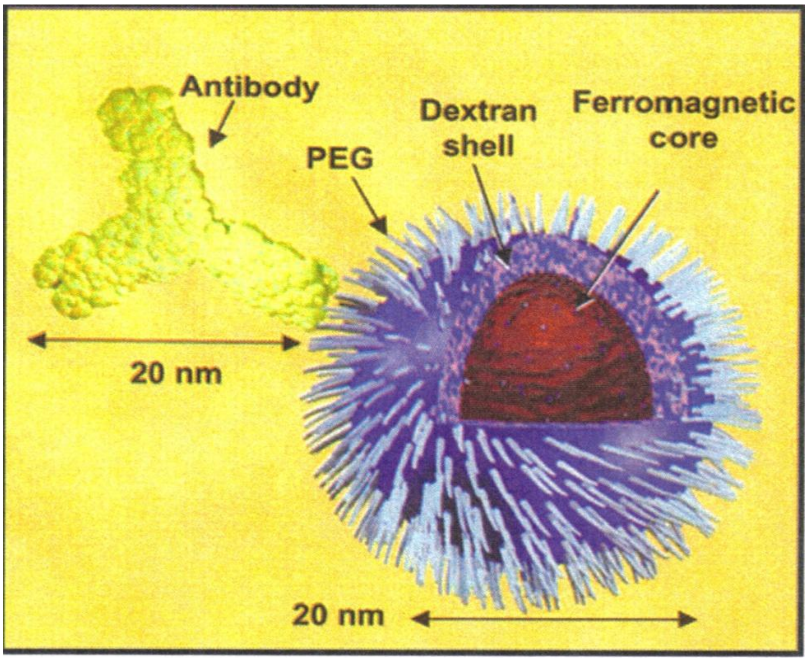


Figure 6

Quantum Dots(Qdots)

Dr.Bawendi$^{(4)}$ discovered that nanocrystals or Qdots are semi-conductor crystals that glow when they are stimulated by ultra-violet light. The wavelength of colour of light emitted from Qdots depends on their size(fig7). Apart from other uses, they are used for imaging and drug therapy in medicine.

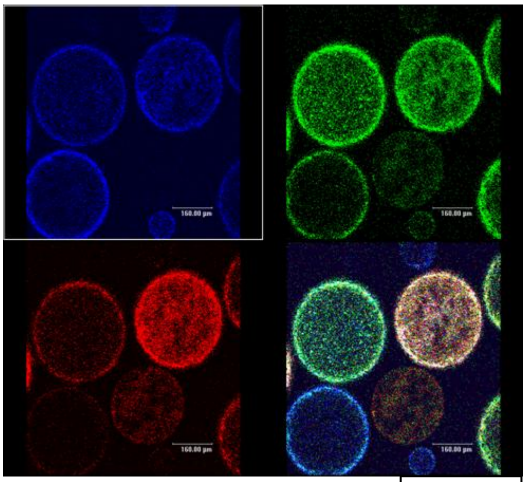


Figure 7

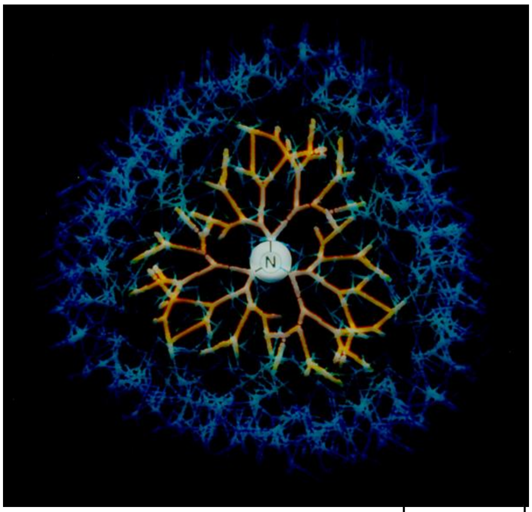


Figure8

Dendrimers

Dendrimers are macromolecular structures that comprise of a series of branches around an inner core(fig8). Dendrimers can encapsulate therapeutic agents within their core as well as attach cell identification tags, fluorescent dyes, enzymes and other molecules onto the ''hooks'' present on their surface(5). Dendrimers are used in combination or diagnostic therapy.

The first nanomedicines are already bringing benefit to thousands of patients(6) There are some applications of nanotechnology already in routine (fig9) use like for e.g., pregnancy tests using gold nanoparticles, sunscreens containing nanoparticles of zinc oxide and others. The first nanotechnology-based cancer drugs have passed regulatory scrutiny and are already on the market.

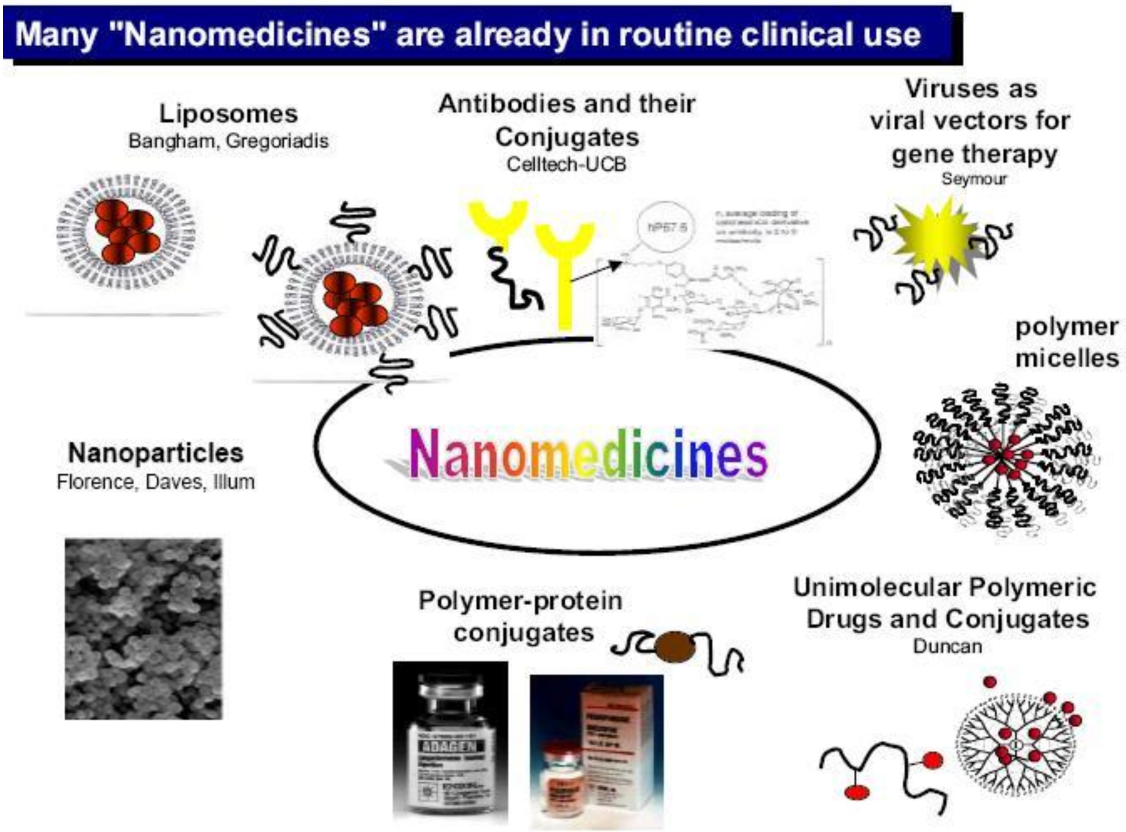


Figure 9

Potential applications of nanotechnology in medicine spans across a variety of areas and can be broadly divided into applications in nanomedicine and applications in nanosurgery(7).

Application of nanorobots in medicine

Potential applications(fig10) of nanorobots include early diagnosis and targeted drug delivery for cancer, monitoring of diabetes and health care.

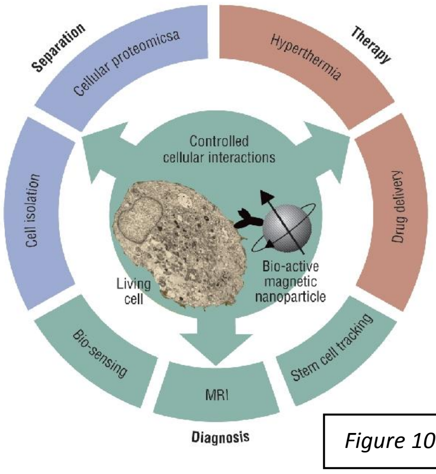


Figure 10

In nanodiagnostics, the ultimate goal is to identify disease at the earliest stage possible, ideally at the level of a single cell (fig11).

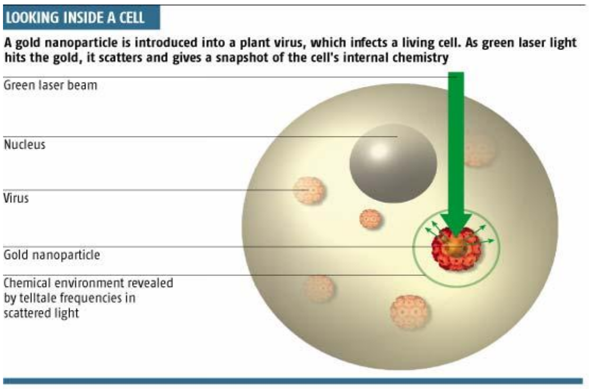


Figure 11

Application in diagnostics

Imaging grade nanoparticles can guide more contrast agent to specific tissue, making small tumours encountered in early stage disease more visible(8). Drugs/imaging agents can be carried by magnetic nanoparticles, which are then injected into the bloodstream. Magnetic fields are focused over the target site allowing guided accumulation of particles which then extravasate at the target site and are later picked up by imaging with MRI or ultrasound. Magnetic field strength falls off rapidly with distance making sites deeper within the body more difficult to target unlike sites closer to the surface. To overcome this problem, some groups have proposed implanting magnetic materials near the target site, within the body(9)

Application in drug delivery and therapy

Nanoparticles can deliver drugs/medicine into specific parts of the human body, thereby making them more effective and less harmful to other parts of the body(10).

Gold nanoshells are useful to fight cancer because of their ability to absorb radiation at certain wavelength. Once the nanoshells enter tumour cells and radiation treatment is applied they absorb the energy and heat up enough to kill the cancer cells.

Application in Gene therapy

Genetic therapies applied at molecular level, involve an artificial ring of DNA designed to enter a cell and up-regulate the expression of genes beneficial to repair e.g., growth factors.

Application in stem cell therapy

Nanoparticles may prove effective tools for improving stem cell therapy. After implantation of stem cells into a living organism, cells may not continue to renew tissue effectively enough to keep the tissue alive long-term. Magnetic nanoparticles offer a simple and robust alternative to viral vectors used(11) and can be used to carry the performance enhancing gene that promotes the growth in the tissue. This technique can be utilised in patients needing transplantations, cardiovascular problems, neurological problems etc.

Application in regenerative medicine

Tissue engineering makes use of artificially stimulated cell proliferation by using suitable nanomaterial-based scaffold and growth factors. The nanoscaffold (12) serves as a guide for the cells to adhere to as they replicate. Nanoscopic fibres can stimulate stem cells at the target site restoring vision, repairing damaged human organs, joints etc.

Application in nanosurgery

A surgical nanorobot, guided by a human surgeon, could act as a semi-autonomous on-site surgeon inside the human body. Such devices could search for pathology, diagnose and then correct lesions by nanomanipulation (13). The instruments would be precise and accurate, targeting only the specific area instead of damaging a large amount of the body. Nanocameras can provide better visualisation of the surgery, reducing the chance of any mistakes or faults. Nanosurgery can also be done at tissue, genetic and cellular levels.

With the design of ‘theranostic’ molecules, nanoparticles could act as one-stop tools to simultaneously diagnose, monitor and treat a wide range of common diseases and injuries and also provide real-time imaging of therapy response in-vivo. The focus of this paper is to explore the possibility of theranostics in cancer management.

Discussion

The use of nanotechnology in cancer treatment offers some exciting possibilities, including the possibility of destroying cancer tumours with minimal damage to healthy tissue and organs, as well as the detection and elimination of cancer cells before they form tumours. Before discussing theranostic approach in cancer management, it is important to discuss both the entities separately.

Cancer

Cancer is a group of diseases in which cells grow and divide abnormally. Cancer cells differ from normal cells in that they can divide forever and live forever by maintaining their telomeres (a region of repetitive DNA at the end of a chromosome, which protects the end of the chromosome from deterioration) **(14)**. Until recently, cancer treatment has been a shotgun approach by administering drugs that reach the malignant cells to destroy them but also distributes all over the body, causing serious side effects and sub-optimal therapeutic response. Over recent decades, there has been explosive development of a variety of nanotechnology platforms, like liposomes, dendrimers etc, to diagnose and treat cancer **(15)**.

Nanotechnology in cancer treatment

Nanoparticles have the advantage of simply being accumulated and entrapped in tumours (passive targeting), called the 'enhanced permeation and retention effect' caused by leaky angiogenetic vessels and poor lymphatic drainage and has been used to explain why NP are found at higher ratios in tumours compared to healthy tissue cells. Attachment of Polyethyleneglycol coating to the nanoparticle protects it from the uptake by Reticulo-Endothelial System and intracellular drug release. By grafting bio-recognition molecules (ligands) onto the nanoparticle, they are recognised by cell surface receptors which lead to receptor-mediated endocytosis (active targeting)**(16)**; use of antibodies to molecules that are expressed on particular cell types, can also allow for cell-specific targeting. For active therapy, nanoparticles are coupled with ligands and chemotherapy agents, which are released at the target site or when subjected to hyperthermia, 'cook' the cancer cells**(17)**.

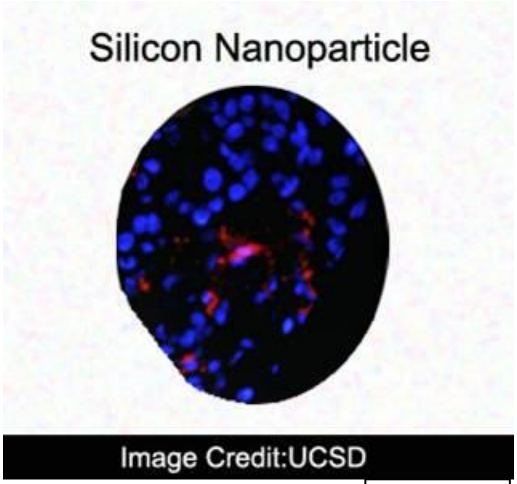


Figure 12

In relation to cancer, there are two main areas in which nanoparticles are being explored: molecular imaging and therapy(18).

In molecular imaging, nanoparticles injected into body are taken up by cancer cells, which allow cancer cells to be specifically imaged(fig12).

Tumour core

As solid tumours grow, they can outgrow their blood supply, resulting in a hypoxic, semi-necrotic tumour core. The well vascularised regions of the tumour are accessible to intravenously administered chemotherapy drugs that may destroy this part of the tumour. However the core is unaffected due to lack of blood supply and in it resides the dormant tumour cells which can recruit macrophages to the core. These macrophages rebuild the blood supply and encourage tumour growth. Muthana(19), exploited this process by loading human macrophages with magnetic nanoparticles (mNP) and placing magnets near the site of a human prostate tumour grown in mice. The macrophages carrying a reporter gene invaded the tumour at a rate more than three times that of the non-loaded cells. As the macrophages are loaded with mNP, they can then be destroyed by hyperthermia after delivering the therapeutic drug or gene.

Disseminated cancer

In disseminated cancer, nanoparticles need to get into areas of the tumour without being taken up by other organs, which is a difficult task, to evade everything in the body except cancer cells. Other researchers have adopted an ‘inverse targeting’ approach by designing nanoparticles that bind only to normal as opposed to cancer cells(3). Tumour cells without the nanoparticles show up as relatively bright objects in MRI.

Theranostics

Theranostics technology(20) measures the activity of biomarkers in diseases, which can vary from one individual to another, enabling efficient and effective drug development. It also enables physicians to tailor optimised therapies to their patients based on the biomarker profile of each individual patient’s specimen, providing safer and more effective therapies by reducing side-effects and increasing tissue bioavailability.

Although there is a considerable amount of research still needed, once developed, this approach should reduce treatment costs and improve patient response to treatment. Today’s biomarker is tomorrow’s theranostic(fig13).

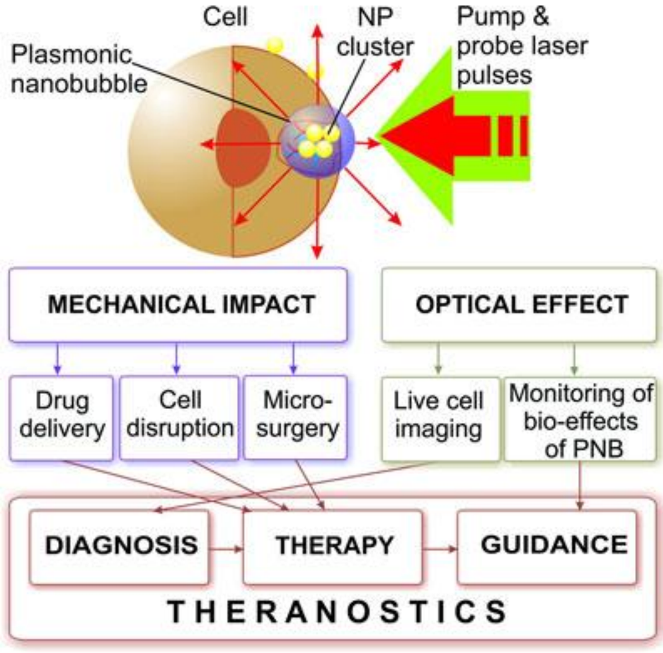


Figure 13

Theranostics in cancer management

Over recent decades, there has been explosive development of a variety of nanotechnology platforms to diagnose and treat cancer. **(21)**

''It will be an advantage to use the same tool to not only locate and identify the cancer, but also to treat it'' Michalet said. 'This technology is likely to explode in the very near future.'**(22)**

Cancer cells that do not respond to conventional therapy can regrow. Tumour heterogenecity and adaptive resistance are the challenges to therapy(20). Cancer treatment should address these two properties of cancer and nanomedicine has the potential to do this, as it has the advantage of being able to target multiple tumour markers and deliver multiple agents simultaneously with minimal toxicity. Theranostics combines diagnostic test with targeted therapy but combining with monitoring of response to therapy will help control this complex disease called cancer (fig14).

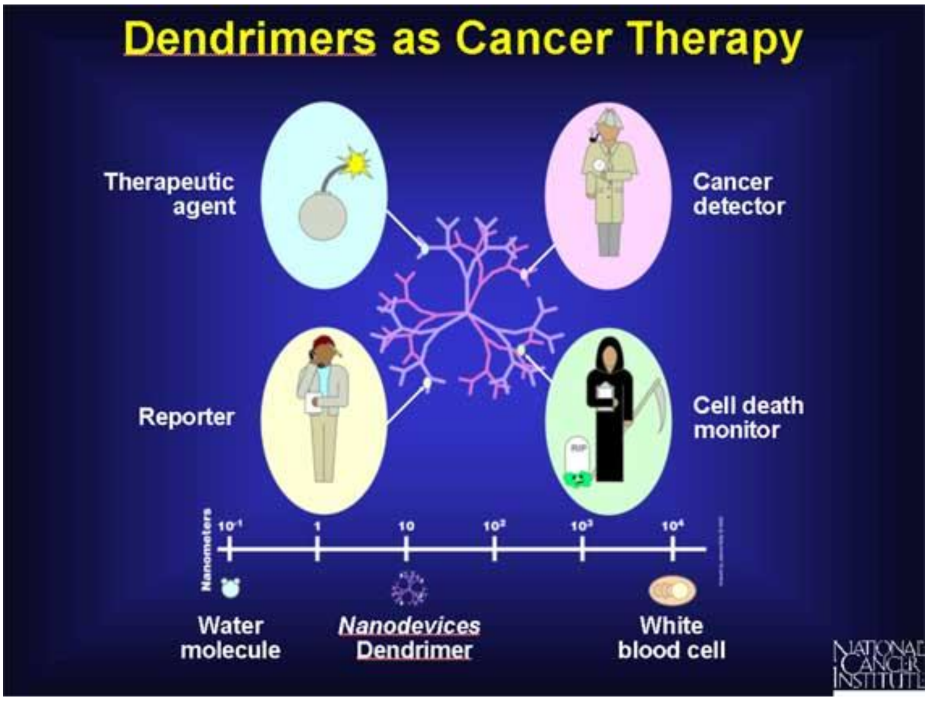


Figure 14

In theranostics, molecular diagnostics by imaging can be first used to locate the tumour to guide target-specific therapy. Because cancer is a heterogenous disease, treatment will probably have to be diverse. Since tumour inevitably evolves in response to treatment, molecular analysis of tumour must be repeated quickly and treatment modified and targeted accordingly. Agents that target molecular markers unique to cancer cells, like monoclonal antibodies(23) have shown to reduce systemic toxicity by achieving targeted therapy by synergistic effect achieved with a single nanomedicine formulation. This would reduce the problem of multidrug administration. Imaging can be used to track nanoparticles systemically, pre-validate appropriate targeting and track the expression pattern of surface markers for adaptive targeting, as well as provide real-time information on tumour response.

Ideally a nanomedicine platform should be small in size, provide high drug-loading capacity, be efficient in targeting to the tumour tissues with minimal non-specific uptake, provide responsive release mechanisms to improve drug bioavailability and also imaging ultrasensitivity to pre-validate and monitor therapy non-invasively. Nanoparticles such as Qdots, which emit light of different colours depending on their size, could enable the simultaneous detection of multiple markers.

It may be possible to develop nanoparticles which are biodegradable or composed of naturally occurring substances which can be eliminated from the body through the natural mechanism of metabolism and excretion. Alternatively, nanoparticles could have a “homing” device which would allow them to be collected and removed after performing the desired function.

The most well-known example in theranostics is Herceptin, the breast cancer drug that targets the HER2 protein. Diagnostic tests are used to detect susceptible tumours, which allow treatment to be limited to patients most likely to benefit from the drug.

One of the on-going researches looks at treating pancreatic cancer. A seamless integration of multiple imaging and therapeutic technologies within a single nanoparticle is required to tackle diseases like, pancreatic cancer which often resist conventional therapies. At the heart of the particle is a nanoshell that can be used to kill cancer cells with heat. The particle can also be tagged with antibodies that allow it to home in on specific types of cancer cells. In addition, the nanoparticles are designed to provide high-resolution images regarding its location in the body by combining a dye for fluorescence imaging with a MRI. These combined capabilities allow researchers to track the nanoparticles throughout the body and even observe their distribution within the tumour before, during and after treatment. This same technology can be applied to treat other forms of cancer.

Ethical Issues

The ethical issues involved with nanomedicine are related to somatic-cell versus germ-cell line therapy, the enhancement of human capabilities, research into human embryonic stem cells, risk assessment in general, and the toxicity, uncontrolled function and self assembly of nanoparticles.

General ethical principles for medicine(24) are, respect for autonomy (informed consent), beneficence (to prevent harm/promote good), nonmaleficence (obligation not to inflict harm), and justice (the question of who should be offered treatment).

Gene therapy

Gene therapy needs to be differentiated between somatic and germline cell therapy. By using gene therapy on germline cells the genetic changes not only affect the individual treated, but also his/her offspring, so if mistakes occur they are irreversible. The obligation to protect future generations and human rights must be respected. Therapy can be used to cure, like for example, in in-utero gene therapy for homozygous alpha-thalassaemia.

Genetic enhancement

It may be possible through nanomedicine to provide genetic enhancement and create “superhuman” capabilities. A line has to be drawn between gene therapy and enhancement and stop “playing God.” The goal of therapy is to treat an existing disease, while the goal of enhancement (improving capacities such as intelligence, longevity, memory etc) is to exceed the boundaries of normalcy and health. Issues of justice like, who should be offered the treatment if it is possible to improve normal characteristics? Will be difficult to answer and justify.

Embryonic stem cell therapy

The ethical issues in stem cell research depend on the source of the stem cells. For somatic stem cells, general ethical principles apply. But use of embryonic stem cells for tissue engineering and cloning raises specific ethical issues. In most cases, human embryonic stem cells are derived from excess embryos following IVF treatment. This becomes an ethical issue as these embryos can develop into human beings. One solution to get around this problem is to use cells isolated from aborted foetuses.

Toxicity

Quantum dots have been shown to be cytotoxic under certain conditions and carbon nanotubes have demonstrated some genotoxicity, possibly due to their nanoscale size (25). , Although the DNA breakages caused by the nanoparticles do not necessarily mean a substance is cancer-causing, it is widely accepted that chemicals causing DNA damage are highly likely to promote mutations leading to cancer. Nanoparticles used in drug delivery may get out of control in the absence of feedback mechanism to control their function (10) and may attack good cells and deliver cargo to wrong parts. Although some of the nanosystems used in drug delivery may be pre-manufactured, Drexler(26) describes the fear of the uncontrolled spread of self-assembling nanoparticles

Societal Implications

Implications are public trust and transparency in relation to new technologies, and the question of whether only the developed countries and the rich will gain from nanotechnology. There could be a value conflict with spending one’s economic resources to obtain advanced treatments to what society accepts as ethically acceptable. Unrestricted freedom of some may endanger the health and safety of others. There may be an increased demand for imaging asymptomatic people concerned they may get cancer.

Economic implications

Governments around the world are investing billions for research in nanotechnology. Research can be quite prolonged, needing more and more funds, although expensive now, theranostics is likely to be more cost effective in the long run if they increase a drug's efficacy or prevent side effects.

Bioterrorism

Nanomedicine could be manipulated to harm the human body rather than healing it. Nanotechnology could tiny nuclear weapons that do not qualify as weapons of mass destruction. A new article in Miami Herald(27) raises a terrifying prospect for nanotech warfare.

<u>Environmental issues</u>

Nanomaterials used need to be non-toxic and eco-friendly; rigorous assessment of these properties is critical to the development of nanomedicine. If the production is not suitably planned, large quantities of nanoparticles could be emitted locally in wastewater and exhaust gasses. Environmental degradation would sharply increase to extremely high levels because nanomaterials would be manufactured in large volumes.

<u>Issues with training and health care resources</u>

New technologies or treatments will be introduced and old got rid of. Unless there is clear strategic research agenda, there will be fragmentation in nanomedicine research. Nanomedicine may be restricted to specialised centres and there will be a need to keep up with the demand for use. Training will be needed to create nano-specialists and patient education. On the other hand, many functions performed on one device hence can be cost effective. Possible nano-monitoring from patient’s home via wireless technology can free up beds in the hospital.

Although nanomedicine raises ethical issues that are more complex than those raised by existing technologies, a reasonably sound knowledge base has already been acquired in the field of bioethics that can be extended to nanomedicine(28).

Conclusion

This paper believes that the multifunctional nanomedicine would achieve synergistic targeted therapy by blocking multiple receptors, reduce systemic toxicity by minimising chemotherapeutic drugs in systemic circulation and achieve further synergy by combining these drugs with molecular targeting and deliver the targeted chemotherapeutic agent specifically to tumours, increasing bioavailability. Widespread adoption of theranostics will eliminate unnecessary treatment of patients for whom the treatment is ineffective or even dangerous, resulting in major drug cost savings for the patient and the healthcare industry. Nanotherapy can also increase life expectancy.

To conclude, my paper has shown that the best form of treatment for cancer in the future is by the theranostic approach. This is personalised medicine which targets the cancer effectively for that individual, not only detects and treats but also monitors the response. Nano theranostics can also be applied for disseminated cancer and to target the tumour core where there is potential for the tumour to regrow. Theranostic approach certainly holds promise for the future in cancer treatment.

Personalised medicine has the potential to revolutionalise the practice of medicine, as it not only tailors the right drug, for the right person at the right time but also evaluates predisposition to disease sometimes decades in advance.

This research has shown me that Successful development of theranostic nanomedicine requires significant advances in imaging and nanomaterials. There is considerable research needed prior to application of nanoparticles for diagnosis and therapy, especially with issues around safety and ethics. Advances in nano-platform, nano-imaging and nano-drug delivery will drive the future development of nanomedicine, personalised medicine and targeted therapy. I support all aspects of research around this 21st century medicine.

After being injected into the body, nanorobots the size of bacteria, might one day roam people's bodies, rooting out disease organisms and repairing damaged tissue. The successful development of a medical nanorobot will change the world of medicine forever. The future being 'personalised medicine'. A completely integrated health system for the 21st century must recognise that in designing drugs and therapeutic programmes, one size does not fit all!

References

1. http://metamodern.com/2009/12/29/theres-plenty-of-room-at-the-bottom%E2%80%9D-feynman-1959
2. **https://ozone.scholarsportal.info/bitstream/1873/14592/1/272679.pdf**
3. **http://www.understandingnano.com/nanoparticles.html**
4. **http://www.invitrogen.com/site/us/en/home/brands/Molecular-Probes/Key-Molecular-Probes-Products/Qdot/Technology-Overview.html**
5. **http://www.actabp.pl/pdf/1_2001/199-208.pdf**
6. **http://www.physorg.com/news1369.html**
   http://www.understandingnano.com/nanoparticles.html
7. **http://www.understandingnano.com/nanoparticles.html**
8. **http://www.understandingnano.com/nanomedicine-nanoparticles-targeted-chemotherapy.html**
9. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2527670
10. Haberzettl CA. (2002) Nanomedicine: destination or journey? *Nanotechnology.*;13(4):R9–R13
11. Mark R Pickard, Perrine Barraud, Divya M Chari (2010)*The transfection of multipotent neural precursor/stem cell transplant populations with magnetic nanoparticles*, j.biomaterials, 1-11.
12. **http://www.sciencedaily.com/releases/2006/09/060915204549.htm**
13. http://www.nanomedicine.com/Papers/IntlJSurgDec05.pdf-
14. http://www.howcurecancer.com/age-cancercells.htm
15. http://www.utsouthwestern.edu/utsw/facdir/cma/fileuploads/8/facdir76088/TheranosticNanomed.pdf
16. **http://www.pnas.org/content/107/3/1235.abstract**
17. http://nano.cancer.gov/learn/impact
18. http://cancergrace.org/cancer-101/2009/07/22/nanoparticles-in-cancer-treatment/
19. Muthana M et al (2008) *A novel magnetic approach to enhance the efficacy of cell-based gene therapies*: Gene Ther. **15** 902-10
20. http://www.utsouthwestern.edu/utsw/facdir/cma/fileuploads/8/facdir76088/TheranosticNanomed.pdf
21. Peer D, Karp JM, Hong S, Farok Hzad OC, Margalit R, Langer R (2007) Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol.*2, 751-760
22. **http://www.understandingnano.com/quantum-dots-locating-cancer-tumor.htm - michaelet**
23. http://www.mrc.ac.uk/Achievementsimpact/Storiesofimpact/Therapeuticantibodies/index.html
24. **http://www.nurse-prescriber.co.uk/Articles/Ethical_prescribing.htm**
25. Nel A, Xia T, Mädler L, Li N. (2006) Toxic potential of materials at the nanolevel. *Science.*; 311(5761):622–627.PubMed

26. Drexler E. (1986) *Engines of Creation: The Coming Era of Nanotechnology.* New York, NY: Anchor Press;
27. http://io9.com/#!5377752/nanotech-could-make-nuclear-weapons-much-much-tinier
28. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1779503

Figures

1. http://nano.gov
2. http://www.bing.com/images/search?q=buckyball&view=detail&id=F734B231FAA70BD900EE9D139B16E68147297E21&first=1&FORM=IDFRIR
3. http://www.bing.com/images/search?q=nanotubes&form=QBIR&qs=n&sk=&sc=2-9
4. http://xnet.rrc.mb.ca/davidb/nanomedicine.htm
5. http://xnet.rrc.mb.ca/davidb/nanomedicine.htm
6. (http://www.bing.com/images/search?q=magnetic+nanoparticles&FORM=BIFD)
7. http://www.bing.com/images/search?q=quantum+dots&form=CPDMHP&mkt=en-gb&qs=n&sk=&sc=2-11&pc=CPDTDF
8. http://www.bing.com/images/search?q=dendrimers&form=QBIR&qs=n&sk=&sc=1-10
9. http://www.bing.com/images/search?q=diagram+for+nanomedicine+already+in+clinical+practice&form=QBIR&qs=n&sk=
   -nanomed in practice
10. http://www.bing.com/images/search?q=nanoparticles&view=detail&id=6282A6725ED91A12A9C98BBCBFFFD9EC431277CE&first=1&FORM=IDFRIR
11. http://xnet.rrc.mb.ca/davidb/nanomedicine.htm
12. http://www.bing.com/images/search?q=silicaon+nanoparticles+image+credit+UCSD&form
13. http://www.bing.com/images/search?q=theranostics&view=detail&id=391FBD1E54982F7E0B57263921A3FD3BA46541EE&first=1&FORM=IDFRIR