International Journal of Biomedical Research

ISSN: 0976-9633 (Online)
Journal DOI:10.7439/ijbr
CODEN:IJBRFA

Review Article

Novel role of nanotechnology in medicine

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Abstract

Nanotechnology by definition means the use of nanoparticles in the field of medicine and for other benefits of mankind. At present, current modalities for diagnosis and treatment of certain diseases, especially for cancer face major limitations, like poor sensitivity and specificity, and drug toxicity respectively. Newer and improved methods based on nanoparticles are being developed. These are used as contrast agents, fluorescent materials, molecular research tools and drugs with targeting antibodies. Nanoparticles used for diagnostic and therapeutic purposes include paramagnetic nanoparticles, quantum dots, nanoshells, nanosomes and dendrimers. Highly toxic anti-cancer chemotherapeutic drugs can be administered with a much better safety profile using drugs with targeting antibodies. Other modalities of nanomedicine are also being developed, which include heat induced ablation of cancer cells by nanoshells and gene therapy. This review article discusses the various platforms of nanotechnology in the field of diagnostics and therapeutics. The potential toxicities of nanoparticles are also described. The safety of nanotechnology is not yet fully defined. So formulation of a new nanodrug faces many regulatory challenges. But definitely, nanotechnology is hoped to play a very crucial role in future medicine.

Keywords: Nanotechnology, nanoparticles, diagnosis, treatment, cancer, drugs

1. Introduction

Nanotechnology can be defined as the science and engineering involved in the design, synthesis and application of drugs and devices on nanometer scale. Its applications have revolutionalised the field of medicine in many aspects. Nanoparticles of size ranging between 1-100 nm are designed and used as biomedical tools of research, for diagnostics and therapeutics.[1] Nanotechnology works from the molecular level using engineered devices and nanostructures, ultimately to achieve medical benefits.

2. Current status of therapeutics

At present, anti-cancer drugs formulations have poor cell specificitty and high toxicity like bone marrow suppression, renal toxicity, hair loss, cardiomyopathy and many other side-effects. Similarly, treatment of insulin-dependent diabetes mellitus faces major challenges of route of drug delivery and achievement of adequate glycemic control. Development of a suitable drug delivery device which could provide non-parenteral dosage form of insulin would be a breakthrough in medicine. The tools of nanotechnology permit a control over the different properties of drugs, such as highly specific site-targetted delivery, controlled release over short or long duration and finally, alteration in solubility and blood pool retention time of drugs.[2] Hence, nanotechnology has enabled designing of drugs with greater degree of cell specificity, greater efficacy and with minimized adverse effects.

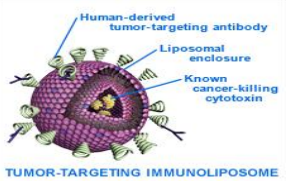
Also, nanotechnology has extensive role in other modalities of cancer treatment, diagnostics, biosensors and many other tools in the field of molecular biology. Many nanotechnology platforms like liposomes, nanobubbles, nanosomes, nonporous, nanoshells, magnetic nanoprobes and nanotubes have been developed.

2.1 Liposomes

Liposomes discovered in mid 1960s are the models of nanoscaled drug delivery devices. These are spherical nanoparticles made of lipid bilayer membranes with an aqueous interior. They can be used as safe and effective drug delivery devices, especially for toxic drugs like anti-cancer drugs and amphotericin-B. The water soluble drugs are loaded in aqueous compartment while lipid soluble drugs are packed in lipid bilayer.[3]

Liposomes can be targeted to a specific organ or tissue by active or passive methods. Vascularity of a tumour tissue is poorly organized and significant leak occurs from blood vessel into the tumour tissue. So the liposomal drug gets accumulated into the tumour tissue passively to produce enhanced effects. Active targeting of the liposomal drug can be achieved by using immunoliposomes or ligand directed liposomes. Immunoliposomes are liposomes conjugated with an antibody directed against the tumour antigen. The antibody can be conjugated to the surface of a stealth liposome, the polyoxyethylene coating of a stealth liposome or on the surface of a non stealth liposome. These immunoliposomes when injected into the body, reaches the target tissue and gets accumulated at its site of action. This reduces unwanted effects and also increases the drug delivery to the target tissue, thus increasing its safety and efficacy. The ligand bearing liposomes are prepared by conjugation with specific ligands directed towards target tissues. For example, ovarian cancer cells have over expression of folate receptors. So the liposomal drug can be conjugated with folate so as to direct the anti-cancer drug molecule to the tumour.[4]

Figure 1: Immunoliposome



2.2 Nanobubbles

Anti-cancer drugs can also be incorporated into the nanobubbles. The nanobubbles can be targeted to the tumour tissue and deliver the drug selectively under the influence of ultrasound exposure. It also enables additional benefit that tumour can be visualized by means of ultrasound.[5,6]

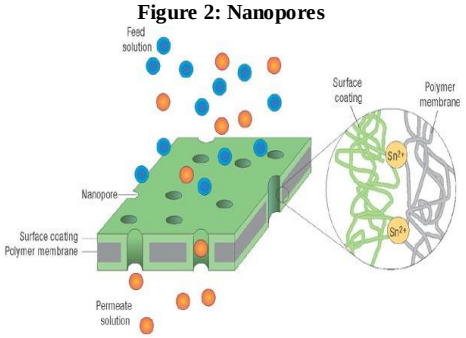
2.3 Nanosomes

Nanosomes have been developed for treatment of various tumours, particularly CNS tumours. Silica coated iron oxide nanoparticles are coated with polyethylene glycol,[7] and affixed with targeting antibody and contrast elements like gadolinium. These are then used to access specific regions of brain involved with tumour. Targeting helps in binding the nanoparticles specifically to the tumour cells, and the contrast elements aid in better detection with magnetic resonance imaging. Subsequent treatment with laser can then destroy the cells loaded with these nanoparticles by the heat generated by iron oxide nanoparticles by absorbing the infra red light.

2.4 Nanopores

Nanopores were designed by Desai and Ferrari in 1997.[8] These consist of wafers with high density of pores(20nm in diameter). The pores allow entry of oxygen, glucose and other nutrients to pass through. On other hand, these do not allow immunoglobulins and cells to pass through them. Nanopores can be used as devices to protect transplanted tissues from the host immune system, while simultaneously using the benefits of transplantation. The beta cells of pancreas can be enclosed within the nanopore device and implanted in the recipient's body. The tissue sample would receive the nutrients from the surrounding tissues, and at the same time remains undetected by immune system and hence do not get rejected. This could serve as a newer modality of treatment for insulin dependent diabetes mellitus.[9]

Figure 2: Nanopores

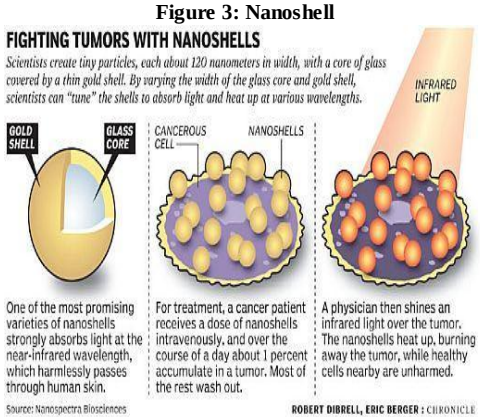


2.5 Nanoshells

Nanoshells consist of nanoparticles with a core of silica and a coating of thin metallic shell. Hirsh *et al*[10] used nanoshells which are tuned to absorb infrared rays when exposed from a source outside the body to demonstrate the thermoablative property of nanoshells. The nanoshells when exposed to NIR (Near Infra Red) region of the electromagnetic spectrum, get heated up and cause destruction of the tissue. The nanoshells are directed to the tumour tissue by immunological methods. With an infrared laser, these are made to get heated up. The polymer melts and releases the drug at the tumour tissue. Nanoshells are currently being investigated for micrometasis of tumours and also for treatment of diabetes mellitus.[11,12]

Gold nanoshells can also detect immunoglobulins at a concentration range of nanograms per milliliter in plasma and whole blood.[11]

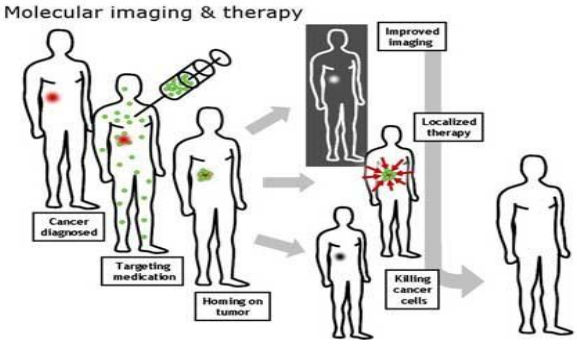
Figure 3: Nanoshell



2.6 Nanoprobes

Magnetic nanoprobes are used for cancer therapy. Iron nanoparticles coated with monoclonal antibodies directed to tumour cells can be made to generate high levels of heat after they accumulate at their target site by external application of alternating magnetic field. This heat kills the cancer cells selectively. This method has been designed by Triton Biosystems and entered clinical trials in 2009.[13] Nanoprobes also allow detection of tumour markers even at molar concentration.[14]

Figure 4: Functions of a nanoprobe



2.7 Nanotubes

Carbon nanotubes discovered in 1991 are tubular structures like a graphite sheet rolled into a cylinder capped at one or both ends by a bucky ball.[15] Nanotubes can be single walled carbon nanotubes(SWCNT) or multiwalled carbon nanotubes(MWCNT) in concentric fashion. These vary in their length ranging from one to few micrometer.[16] Nanotubes have greater strength and stability and hence, can be used as stable drug carriers. Cell specificity can be achieved by conjugating antibodies to carbon nanotubes with fluorescent or radiolabelling.[17] Entry of nanotubes into the cells may be mediated by endocytosis or by insertion through the cell membrane.

Amphotericin B nanotubes have shown increased drug delivery and efficacy into the cells compared to Amphotericin B administration without nanotubes.[18] And these nanotubes were found to be effective on strains of fungi which are usually resistant to Amphotericin B alone. Further, there was reduced toxicity to mammalian cells with Amphotericin B nanotubes.

Nanotubes can also transport DNA across cell membranes and hence can be used in gene therapy. DNA can be attached to the tips of nanotubes or can be incorporated within the nanotubes. Prato *et al*[18] showed greater expression of beta-galactosidase marker gene through nanotubes compared to naked DNA. This confers the advantage of non-immunogenicity in contrast to viral vectors used for gene transfer.

2.8 Dendrimers

Dendrimers are nanomolecules with regular branching structures. The number of branching determines the size of the dendrimer which can be controlled. The branches arise from the core in shape of a spherical structure by means of polymerization. This results in formation of cavities within the dendrimer molecule which can be used for drug transport. The ends of the dendrimer molecule can be attached with other molecules for transport. These molecules give the dendrimers various functional applications.[19]

Tectodendrimers are complexes of dendrimers, with each dendrimer module of the complex performing different functions such as targeting, diagnosis of disease state, delivery of drug and imaging. This extended nanodevice has potential applications in cancer chemotherapy as a mode of targeted drug therapy.[20]

Dendrimers can be used for gene therapy where these can replace conventional viral vectors. They enter the cells by endocytosis and the DNA gets transported into nucleus for transcription of the applied gene. The advantage of dendrimer based therapy is absence of stimulation of immune reaction. Dendrimers have been tested in mammalian cell types and in animal models. Huang *et al*[21] have demonstrated the potential use of transferring conjugated PEG modified polyamidoamine (PAMAM) dendrimers for targeted gene delivery to the brain.

NanoJuice Transfection Kit produced by EMD Chemicals Inc. and Superfect® Transfection Reagent of Qiagen are dendrimer based DNA transfection kits used for delivering DNA into the cell. These are claimed to have improved transfection efficacy and low toxicity to cells.[22,23]

Dendrimers are also used as contrast agents for imaging. The 1,4-diaminobutane (DAB) core dendrimer and the PAMAM dendrimer are well studied commercially available dendrimers for imaging studies. Renal excretion is the main route of clearance and is dependent on the size of the particle and more than 60 per cent of injected DAB or PAMAM dendrimer is cleared from circulation within 15 min.[23.]

PAMAM dendrimers can also be used in treatment of cancer by conjugating with anti-cancer drugs like cisplatin, adriamycin or methotrexate.[24]

3. Nanotechnology in gene therapy

Gene therapy is a newer modality of approach for treatment of many genetic disorders including diabetes mellitus,[25] cystic fibrosis,[26] and alpha 1 antitrypsin deficiency.[27] Viral vectors used for gene transfer have the limitations of safety concerns and stimulation of immune system with production of antibodies against the viral vectors. Further, the naked DNA used in viral vectors cannot cross the negatively charged cell membrane as these are also negatively charged.[27,28] Liposomes measuring less than 100 nm can be used for effective delivery of genetic material into the cells.

Niu *et al*[25] used human insulin gene in chitosan nanoparticles to transfect Streptozocin induced diabetic rat through gastrointestinal tract. They found a significant decrease in fasting blood glucose level, increase in plasma insulin levels and increased expression of human insulin gene m RNA in the study rats. This study may lead to the development of newer modality of treatment of type 1 diabetes mellitus.

4. Role of Nanotechnology in In-vivo diagnosis

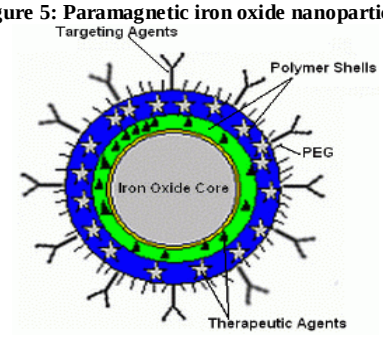
Nanoimaging uses various techniques like para-magnetic iron oxide nanoparticles[29] for the study of in-vivo molecular events and molecules manipulation, particularly by magnetic resonance imaging.

4.1 Para-magnetic nanoparticles

Diagnostically, para-magnetic iron oxide nanoparticles are used as contrast agents in magnetic resonance imaging. These have greater magnetic susceptibility than conventional contrast agents. Targetting of these nanoparticles enables identification of specific organs and tissues.[20] Para-magnetic nanoparticles conjugated with antibodies to HER-2/neu which are expressed on breast cancer cells have been used with MRI to detect breast cancer cells in vitro.[30] Study done by Leuschner *et al*[31] has demonstrated the in vivo detection of breast cancer cells using para-magnetic nanoparticles conjugated with luteinizing hormone releasing hormone as breast cancer cells express LHRH receptors.

Monocrystalline iron oxide nanoparticles (MIONS) have been studied by Knauth *et al*[32] for preoperative MRI of brain. MIONS help to overcome the disadvantage of surgically induced contrast enhancement with traditional contrast agents which can result in misinterpretation during intraoperative MRI of brain. MIONS are rapidly taken up the tumour cells, producing long lasting contrast enhancement of tumour.[33]

Figure 5: Paramagnetic iron oxide nanoparticles



4.2 Role of Nanotechnology in in-vitro diagnostics

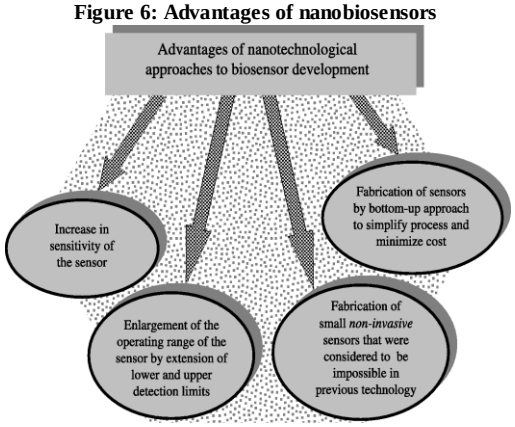
Nanotechnology has found its use in diagnostic medicine as contrast agents, fluorescent dyes and magnetic nanoparticles. Among in-vitro diagnostics, nanotechnology has advanced into two vast application areas: Central analytical laboratories and 'point-of-care' devices. It has enabled development of new generation devices which are smaller, faster and cheaper.[34]These devices do not require special skills and provide accurate readings. These devices need much smaller samples and will deliver more accurate and more complete biological data from a single reading.

'Nanotechnology on a chip' is a new paradigm for total chemical analysis systems.[35] Nanotechnology based biochips and microarrays include nanofluidic arrays, DNA chips and protein nanobiochips. These chips have much higher cell specificity.[36] On other hand, 'cells-on-chips' use cells as their sensing elements.

5. Nanobiosensors

Nanomaterials are very sensitive chemical and biological sensors.[37] Based on differences in volume, concentration, magnetic forces, pressure or temperature in body, nanobiosensors can differentiate and recognize certain cells, particularly cancer cells.

Figure 6: Advantages of nanobiosensors



6. Potential hazards of nanoparticles

Nanoparticles can cause varied pathologies of lungs, gastrointestinal system and other body organs. Intratracheal instillation of carbon nanotubes can cause varied pathologies of lungs like epitheloid granuloma, interstitial inflammation, peribronchial inflammation and necrosis of lung. The risk was found to be greater than that produced by carbon black and quartz.[38]

Radomski *et al*[39] have observed the proaggregatory effects of nanotubes on platelets in in-vitro studies and acceleration of vascular thrombosis in rat. It was also observed that fullerenes do not have the property of inducing platelet aggregation. Thus, for designing nanoparticles based drug delivery systems, fullerenes may be a safer approach as compared to nanotubes.

In gastrointestinal system, nanoparticles can cause inflammatory bowel diseases. The toxicity of nanoparticles may be related to their ability to induce release of pro- inflammatory mediators, leading to inflammatory response and organ damage. If ingested, the nanoparticles can enter circulation and reach different organs and systems, resulting in toxicity.[40]

Nanoparticles have been studied in-vitro and in animal models. Their safe use in humans needs further extensive research and much needed caution.

7. Regulatory challenges with nanomedicines

Regulatory issues play a major role in development of new nanoformulation drugs. A nanoformulation of a drug which is based on a previously approved drug in microformulation can undergo a shorter approval pathway by means of abbreviated new drug application if bioequivalence can be demonstrated to its microformulation drug.

However, if bioequivalence cannot be demonstrated, it would necessitate approval of all the stages of new drug application. Further, when a nanodrug is designed as a new chemical entity, the evaluation procedure becomes more stringent.[41]

Nanodrug manufacturers must comply with FDA's Current Good Manufacturing Practices (CGMP) and Quality System Regulations (QSR).[42] The equipments for nanodrug manufacturing and control of contamination also come under stringent regulation. The drug products are purified by the use of filters and CGMP demands that the filters do not release fibres.[43]

The FDA centres namely, the Center for Drug Evaluation and Research (CDER), the Center for Devices and Radiological Health (CDRH) and the Center for Biologics Evaluation and Research (CBER) regulate drugs, devices and biologics respectively and are responsible for regulating nanomedicinal products. FDA classifies medicinal products as drug, device or biologics according to their primary mode of action to assign a centre for their primary jurisdiction during the evaluation process.

In case of a nanodrug it is difficult to classify it as a drug, device or biologics since it tends to have a combination of the above. Hence, the assignment of the Centre becomes difficult. Further, the drug has to pass through all the Centres of FDA owing to its complexity. This results in greater time period for approval of the drug. The staff of FDA must also be sufficiently educated and trained in nanotechnology in the field of medicine to evaluate nanodrug products.[44]

8. Conclusion

Use of nanotechnology in medicine needs adequate evaluation of its risk and safety factors. However, it is expected that nanomedicine would play a crucial role in diagnosis and treatment of human diseases in future. With concurrent application of nanotechnology in other fields, its utility is likely to extend further into diagnostics, molecular research techniques and tools.

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