**Review Article**

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# Novel applications of nanotechnology in medicine

A. Surendiran, S. Sandhiya, S.C. Pradhan & C. Adithan

*Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education & Research Puducherry, India*

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**Current modalities of diagnosis and treatment of various diseases, especially cancer have major limitations such as poor sensitivity or specificity and drug toxicities respectively. Newer and improved methods of cancer detection based on nanoparticles are being developed. They are used as contrast agents, fluorescent materials, molecular research tools and drugs with targeting antibodies. Paramagnetic nanoparticles, quantum dots, nanoshells and nanosomes are few of the nanoparticles used for diagnostic purposes. Drugs with high toxic potential like cancer chemotherapeutic drugs can be given with a better safety profile with the utility of nanotechnology. These can be made to act specifically at the target tissue by active as well as passive means. Other modalities of therapy such as heat induced ablation of cancer cells by nanoshells and gene therapy are also being developed. This review discusses the various platforms of nanotechnology being used in different aspects of medicine like diagnostics and therapeutics. The potential toxicities of the nanoparticles are also described in addition to hypothetical designs such as respirocytes and microbivores. The safety of nanomedicine is not yet fully defined. However, it is possible that nanomedicine in future would play a crucial role in the treatment of human diseases and also in enhancement of normal human physiology.**

**Key words** Diagnostics - drugs - nanomedicine - nanotechnology - therapeutics

## Introduction

Nanomedicine involves utilization of nanotechnology for the benefit of human health and well being. The use of nanotechnology in various sectors of therapeutics has revolutionized the field of medicine where nanoparticles of dimensions ranging between 1 - 100 nm are designed and used for diagnostics, therapeutics and as biomedical tools for research[1]. It is now possible to provide therapy at a molecular level with the help of these tools, thus treating the disease and assisting in study of the pathogenesis of disease. Conventional drugs suffer from major limitations of adverse effects occurring as a result of non specificity of drug action and lack of efficacy due to improper or ineffective dosage formulation (*e.g.*, cancer chemotherapy and antidiabetic agents). Designing of drugs with greater degree of cell specificity improves efficacy and minimizes adverse effects. Diagnostic methods with greater degree of sensitivity aid in early detection of the disease and provide better prognosis. Nanotechnology is being applied extensively to provide targeted drug therapy, diagnostics, tissue regeneration, cell culture, biosensors and other tools in the field of molecular biology. Various nanotechnology platforms like fullerenes, nanotubes, quantum dots, nanopores, dendrimers, liposomes, magnetic nanoprobes and radio controlled nanoparticles are being developed.

## Current status of therapeutics

The major factors influencing the treatment outcome in a patient are the efficacy and safety profile of the drug more so when used for cancer chemotherapy. These drugs have poor cell specificity and high toxicity like bone marrow suppression, gastric erosion, hair loss, renal toxicity, cardiomyopathy, and several effects on other systems. Similarly treatment for diabetes faces challenges with the route of delivery and inadequate glycaemic control. Availability of non-parenteral dosage forms of insulin would be a breakthrough and development of a suitable drug delivery device can aid in this approach. In many cases, the sensitivity and specificity of various diagnostic methods as in radio imaging and various assays for detection of malignancy are not sufficient enough for early detection and treatment.

## Nanotechnology and medical applications

Development of newer drug delivery systems based on nanotechnology methods is being tried for conditions like cancer, diabetes, fungal infections, viral infections and in gene therapy. The main advantages of this modality of treatment are targeting of the drug and enhanced safety profile. Nanotechnology has also found its use in diagnostic medicine as contrast agents, fluorescent dyes and magnetic nanoparticles (Table 1).

### *Liposomes*

Liposomes discovered in mid 1960s were the original models of nanoscaled drug delivery devices. They are spherical nanoparticles made of lipid bilayer membranes with an aqueous interior but can be unilamellar with a single lamella of membrane or multilamellar with multiple membranes. They

**Table.** Some nanoparticles used for medical applications*

| Study phase | Product | Description | Use | Manufacturer |
|---|---|---|---|---|
| Preclinical | MRX 952 | Nanoparticle preparation – to encapsulate camptothecin analogues | Tumours | IMA Rx Therapeutics |
| Preclinical | Targeted Nano Therapeutics (TNT)™ system | TNT with polymer coated iron oxide magnetic particle | Solid tumours | Triton Biosystems |
| Preclinical | AuroLase™ | Gold nanoshell | Head and neck cancer | Nanospectra Biosciences Inc |
| Preclinical | Dendrimer-Magnevist# | PAMAM dendrimer | MRI imaging agent | Dendritic Nanotechnologies Inc |
| Phase 1 | VivaGel® | Dendrimer based microbicide gel | HIV prevention | Starpharma Pty Ltd |
| Phase 1 | INGN 401 | Nanoparticle formulation of tumour suppression gene FUS1 | Lung cancer | Introgen Therapeutics Inc |
| Phase 1&2 | Cyclosert-Camptothecin – IT 101 | β-Cylcodextrin polymer drug delivery system | Solid tumours | Calando Pharmaceuticals |
| Phase 2 | VivaGel® | Dendrimer based microbicide gel | HSV prevention | Starpharma Pty Ltd |
| Phase 2 | MRX 815 | Nanobubble technology | Treatment of intravascular clot | IMA Rx Therapeutics |
| Phase 3 | Combidex® / Ferumoxtran 10 | Iron oxide nanoparticle | MRI contrast agent | AMAG Pharmaceuticals |
| Marketed | Abraxane® | Albumin bound taxane particles | Non small cell lung cancer | Abraxis Oncology |
| Marketed | AmBisome® | Liposomal preparation of amphotericin B | Fungal infection | Astellas Pharma US |
| Marketed | Doxil® | Liposomal doxorubicin | Ovarian tumour | Ortho Biotech |

* Information obtained from respective company webpage on internet
# Available at Nanotechnology Characterization Laboratory Webpage at *http://ncl.cancer.gov/*

can be used as effective drug delivery systems. Cancer chemotherapeutic drugs and other toxic drugs like amphotericin and hamycin, when used as liposomal drugs produce much better efficacy and safety as compared to conventional preparations. These liposomes can be loaded with drugs either in the aqueous compartment or in the lipid membrane. Usually water soluble drugs are loaded in aqueous compartment and lipid soluble drugs are incorporated in the liposomal membrane[2]. The major limitation of liposome is its rapid degradation and clearance by the liver macrophages[3], thus reducing the duration of action of the drug it carries. This can be reduced to a certain extent with the advent of stealth liposomes where the liposomes are coated with materials like polyoxyethylene[4] which prevents opsonisation of the liposome and their uptake by macrophages[5]. Other ways of prolonging the circulation time of liposomes are incorporation of substances like cholesterol[6], polyvinylpyrollidone polyacrylamide lipids[7] and high transition temperature phospholipids distearoyl phosphatidylcholine[8].

*Targeting of liposomal drugs*: Liposomes can be targeted to specific organ or tissue by passive as well as active methods. As the liposomal drug acts minimally on other tissues, the safety profile is better than non-liposomal drug. The vascularity in tumour tissue is poorly organized and significant leak occurs from blood vessel in the tumour tissue. The liposomal drugs get accumulated in the tumour tissue passively and produce enhanced effects. Active targeting of the drug can be achieved by using immunoliposomes and ligand directed liposomes.

Immunoliposomes are liposomes conjugated with an antibody directed towards 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 in its site of action. This reduces unwanted effects and also increases the drug delivery to the target tissue, thus enhancing its safety and efficacy[9].

Antibody directed enzyme prodrug therapy (ADEPT) consists of liposomes conjugated with an enzyme to activate a prodrug and an antibody directed to a tumour antigen (enzyme linked immunoliposomes). These are administered prior to administration of a prodrug. The antibody directs the enzyme to the target tissue where it activates the prodrug selectively and converts it to its active form. This way, action of the drug is avoided in other normal tissues, thus minimizing the toxicity of drug[10-12]. Such studies are being tried with epirubicin and doxorubicin[11,13].

Ligand bearing liposomes are conjugated with specific ligands which are directed towards target structures. In ovarian cancer, overexpression of folate receptors by the tumour tissue occurs. The liposomal drug can be conjugated with folate so as to direct the molecule to the tumour[14]. This method is also being tried in the treatment of leishmaniasis where liposomal hamycin conjugated with mannosyl human serum albumin are targeted towards human macrophages[15]. Asialofeutin conjugation is being tried to target liver cells for gene therapy[16]. The targeted liposomal preparations are found to have a better efficacy than non targeted liposomes.

***Nanopores***

Nanopores designed in 1997 by Desai and Ferrari[17], consist of wafers with high density of pores (20 nm in diameter). The pores allow entry of oxygen, glucose and other products like insulin to pass through. However, it does not allow immunoglobulin and cells to pass through them. Nanopores can be used as devices to protect transplanted tissues from the host immune system, at the same time, utilizing the benefit of transplantation. β cells of pancreas can be enclosed within the nanopore device and implanted in the recipient's body. This tissue sample receives the nutrients from the surrounding tissues and at the same time remains undetected by the immune system and hence do not get rejected. This could serve as a newer modality of treatment for insulin dependent diabetes mellitus[18].

Nanopores can also be employed in DNA sequencing. Branton's team at Harvard University[19], has been working on modified nanopores that have the ability to differentiate DNA strands based on differences in base pair sequences. Nanopores are also being developed with ability to differentiate purines from pyrimidines. Further, incorporation of electricity conducting electrodes is being designed to improve longitudinal resolution for base pair identification[19]. Such a method could possibly read a thousand bases per second per pore. These can be used for low cost high throughput genome sequencing[20] which would be of great benefit for application of pharmacogenomics in drug development process.

### *Fullerenes*

Fullerenes, a carbon allotrope, also called as "bucky balls" were discovered in 1985[21]. The buckminster fullerene is the most common form of fullerene measuring about 7 Å in diameter with 60 carbon atoms arranged in a shape known as truncated icosahedrons[22]. It resembles a soccer ball with 20 hexagons and 12 pentagons and is highly symmetrical[23].

*Types of fullerenes*: Alkali doped fullerenes are structures with alkali metal atoms in between fullerenes contributing valence electrons to neighbouring fullerenes[24]. They occur because of the electronegative nature of the fullerenes.

Endohedral fullerenes have another atom enclosed inside the buckyball. If a metallic atom is enclosed, these are called as metallofullerenes[25,26]. Due to the small size of $C_{60}$ fullerene, it is difficult to synthesize endohedral $C_{60}$ fullerenes. However, larger fullerenes such as $C_{82}$ or $C_{84}$ fullerenes are used for synthesizing endohedral fullerenes. Endohedral metallofullerenes can be used for diagnostic purposes as radio contrast media in magnetic resonance imaging and other imaging procedures. Since the radioactive metal is enclosed within the buckyball, these are less toxic and safer. This method can also be employed for imaging organs as radioactive tracers[26].

Exohedral fullerenes also called as fullerene derivatives, are synthesized by chemical reaction between the fullerene and other chemical groups. These are also called as functionalized fullerenes. Such fullerenes can be used as photosensitizers in photodynamic therapy for malignancies. These generate reactive oxygen species when stimulated by light and kills the target cells. This method is now also being investigated for antimicrobial property as these cause cell membrane disruption especially in Gram positive bacteria and mycobacterium[27-29].

Heterofullerenes are fullerene compounds where one or more carbon atoms are replaced by other atoms like nitrogen or boron[21].

Fullerenes are being investigated for drug transport of antiviral drugs, antibiotics and anticancer agents[27-30]. Fullerenes can also be used as free radical scavengers due to presence of high number of conjugated double bonds in the core structure. These are found to have a protective activity against mitochondrial injury induced by free radicals[31]. However, fullerenes can also generate reactive oxygen species during photosensitization. This property can be used in cancer therapy[32].

Fullerenes have the potential to stimulate host immune response and production of fullerene specific antibodies. Animal studies with C60 fullerene conjugated with thyroglobulin have produced a C60 specific immunological response which can be detected by ELISA with IgG specific antibodies. This can be used to design methods of estimation of fullerene levels in the body when used for therapeutic or diagnostic purposes[33]. On intravenous injection, these get distributed to various parts of the body and get excreted unchanged through the kidney. Soluble derivates of fullerenes are more biocompatible compared to insoluble forms of fullerenes and have low toxic potential even at higher doses[33]. Further, the degree of purification of fullerene determines its cost and highly purified fullerenes are expensive, restricting its application in medical field[21].

### *Nanotubes*

Carbon nanotubes discovered in 1991[34] are tubular structures like a sheet of graphite rolled into a cylinder capped at one or both ends by a buckyball. Nanotubes can be single walled carbon nanotube (SWCNT) or multiwalled carbon nanotube (MWCNT) in concentric fashion. Single walled nanotube has an internal diameter of 1-2 nm and multiwalled nanotube has a diameter of 2-25 nm with 0.36 nm distance between layers of MWCNT. These vary in their length ranging from 1 μm to a few micrometers[35]. These are characterized by greater strength and stability hence can be used as stable drug carriers. Cell specificity can be achieved by conjugating antibodies to carbon nanotubes with fluorescent or radiolabelling[36]. Entry of nanotubes into the cell may be mediated by endocytosis or by insertion through the cell membrane. Carbon nanotubes can be made more soluble by incorporation of carboxylic or ammonium groups to their structure and can be used for the transport of peptides, nucleic acids and other drug molecules. Indium-111 radionuclide labelled carbon nanotubes are being investigated for killing cancer cells selectively[35].

Amphotericin B nanotubes has shown increased drug delivery to the interior of cells compared to amphotericin B administration without nanotubes[37]. The efficacy of amphotericin B nanotubes was greater as an antifungal agent compared to amphotericin B alone and it was 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[37]. The ability of nanotubes to transport DNA across cell membrane is used in studies involving gene therapy. DNA can be attached to the tips of nanotubes or can be incorporated within the tubes. Prato *et al*[37] showed greater expression of the β galactosidase marker gene through nanotubes compared to transfer of naked DNA. This confers the advantage of non immunogenicity in contrast to viral vectors used for gene transfer. Gene silencing studies with small interfering RNA (siRNA) have been done as a modality of cancer therapy where tumour cells will be selectively modulated. Functionalized single walled carbon nanotubes can be used with siRNA to silence targeted gene expression[38].

It was observed that carbon nanotubes, except acetylated ones, when bonded with a peptide produce a higher immunological response compared to free peptides. This property can be used in vaccine production to enhance the efficacy of vaccines. Further, it was also found that compounds bound to nanotubes increase the efficacy of diagnostic methods like ELISA. These can also be used for designing of biosensors owing to property of functionalization and high length to diameter aspect ratio which provides a high surface to volume ratio[39,40].

Water insoluble forms of nanotubes like pristine carbon nanotubes have high *in vitro* toxicity compared to modified water dispersible forms of nanotubes. It was also seen that the toxic potential decreases with functionalization. Further, functionalization also affects the elimination of the nanotube. SWCNTs without conjugation to monoclonal antibody have a high renal uptake and modest liver uptake as compared to SWCNTs with conjugation to monoclonal antibody having higher liver uptake and lower renal uptake[41].

### *Quantum dots*

Quantum dots are nanocrystals measuring around 2-10 nm which can be made to fluorescence when stimulated by light. Their structure consists of an inorganic core, the size of which determines the colour emitted, an inorganic shell and an aqueous organic coating to which biomolecules are conjugated. The biomolecule conjugation of the quantum dots can be modulated to target various biomarkers[42].

Quantum dots can be used for biomedical purposes as a diagnostic as well as therapeutic tool. These can be tagged with biomolecules and used as highly sensitive probes. A study done on prostate cancer developed in nude mice has shown accumulation of quantum dots probe by enhanced permeability and retention as well as by antibody directed targeting[43]. The quantum dots conjugated with polyethylene glycol (PEG) and antibody to prostate specific membrane antigen (PSMA) were accumulated and retained in the grafted tumour tissue in the mouse[43].

Quantum dots can also be used for imaging of sentinel node in cancer patients for tumour staging and planning of therapy. This method can be adopted for various malignancies like melanoma, breast, lung and gastrointestinal tumours[42]. Quantum dot probes provide real time imaging of the sentinel node with Near Infra Red (NIR) fluorescence system. The NIR region of the electromagnetic spectrum produces reduced background noise and deeper penetration of rays, of up to 2 to 5 cm into the biological sample. However, the traditional fluorescence dyes yield low signal intensity when used in NIR region. This limitation is overcome, by using NIR fluorescence system with quantum dot probes. The fluorescence produced by quantum dots is much brighter than those produced by conventional dyes when used with NIR fluorescence system[44]. However, the application of quantum dots in a clinical setting has limitations owing to its elimination factors. Functionalization of the quantum dots which protects from the toxic core, leads to increase in size of the nanoparticle greater than the pore size of endothelium and renal capillaries, thus reducing its elimination and resulting in toxicity. Also, *in vivo* studies are lacking on the metabolism and excretion of quantum dots[42].

### *Nanoshells*

Nanoshells were developed by West and Halas[45] at Rice University as a new modality of targeted therapy. Nanoshells consist of nanoparticles with a core of silica and a coating of thin metallic shell. These can be targeted to desired tissue by using immunological methods. This technology is being evaluated for cancer therapy. Hirsh *et al*[46] used nanoshells which are tuned to absorb infra red rays when exposed from a source outside the body to demonstrate the thermo ablative property of nanoshells. The nanoshells when exposed to NIR region of the electromagnetic spectrum get heated and cause destruction of the tissue. This has been studied in both *in vitro* and *in vivo* experiments with HER 2 expressing SK-BR-3 human breast carcinoma cells. The control cells did not lose their viability even after treatment with nanoshells with non specific anti IgG or PEG and NIR ablation[47].

Nanoshells can also be embedded in a hydrogel polymer containing the drug. After directing the nanoshells to the tumour tissue by immunological methods, with an infrared laser, these can be made to get heated up, melting the polymer and releasing the drug at the tumour tissue. Targeting the drug release avoids the toxicity of cancer chemotherapy drugs. Nanoshells are currently being investigated for micro metastasis of tumours and also for treatment of diabetes[19,48].

Nanoshells are also useful for diagnostic purposes in whole blood immunoassays. Gold nanoshells can be coupled to antibodies and the size can be modulated so that it responds to NIR wavelength, which has the ability to penetrate whole blood specimens. With this method it is possible to detect immunoglobulins at a concentration range of nanograms per millilitre in plasma and whole blood[19].

***Nanobubbles***

Cancer therapeutic drugs can be incorporated into nanoscaled bubble like structures called as nanobubbles. These nanobubbles remain stable at room temperature and when heated to physiological temperature within the body coalesce to form microbubbles. These have the advantages of targeting the tumour tissue and delivering the drug selectively under the influence of ultrasound exposure. This results in increased intracellular uptake of the drug by the tumour cells. It also provides an additional advantage of enabling visualisation of the tumour by means of ultrasound methods[49,50]. Rapaport *et al*[51] have demonstrated the utility of nanobubbles in delivery of drugs like doxorubicin based on *in vitro* and *in vivo* experiments using breast cancer cells MDA MB231 and mice with breast cancer xenograft respectively. On administration of nanobubble loaded doxorubicin, these reach the tumour tissue through leaky vasculature and get accumulated at the site of tumour. This is followed by formation of microbubbles by coalescing of nanobubbles which can be visualised by ultrasound techniques. When the site is focused with high intensity focused ultrasound (HIFU), it causes disruption of the microbubbles resulting in release of the drug. The microbubbles retained the drug in a stable state until stimulated by HIFU. This results in attainment of higher levels of drug in the target cells and hence reduced toxicity and increased efficacy. This method needs further exploration for its utility in treatment of various malignancies. Liposomal nanobubbles and microbubbles are also being investigated for their role as effective non viral vectors for gene therapy. Nanobubbles combined with ultrasound exposure has shown improved transfer of gene in both *in vitro* and *in vivo* studies[52,53]. Nanobubbles are also being tried as a therapeutic measure for removal of clot in vascular system in combination with ultrasound, a process called as sonothrombolysis. This method has advantages of being non invasive and causing less damage to endothelium[54].

***Paramagnetic nanoparticles***

Paramagnetic nanoparticles are being tried for both diagnostic and therapeutic purposes. Diagnostically, paramagnetic iron oxide nanoparticles are used as contrast agents in magnetic resonance imaging. These have a greater magnetic susceptibility than conventional contrast agents. Targeting of these nanoparticles enables identification of specific organs and tissues[55]. The use of iron oxide in MRI imaging faces limitations like specificity and internalization by macrophages[56]. Paramagnetic 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*[57]. Study done by Leuschner *et al*[58] has demonstrated the *in vivo* detection of breast cancer cells using paramagnetic nanoparticles conjugated with luteinizing hormone releasing hormone as breast cancer cells express LHRH receptors. Thus, use of antibodies to direct the nanoparticle to the target site helped to overcome problems with specificity of action. Internalization of the nanoparticles by macrophages can be reduced by treatment with drugs like lovastatin which reduce macrophage receptor expression for the nanoparticle by reducing the recycling of receptors[56]. Further, injection of decoys of nanoparticle can be used to eliminate plasma opsonins and reduce uptake of the nanoparticles. Also, change of surface charge of the nanoparticle to neutral by covalent coupling to chemicals leads to an increase in circulation time[56].

Monocrystalline iron oxide nanoparticles (MIONs) have been studied by Knauth *et al*[59] in magnetic resonance imaging of brain. MIONs help in overcoming the disadvantage of surgically induced contrast enhancement with traditional contrast agents resulting in misinterpretation during intra-operative MR imaging of brain. Surgically induced contrast enhancement occurs in brain due to leak of contrast material from the cut end and oozing blood vessels in brain when MR imaging is done post-operatively. This is avoided when MIONs are used pre-operatively.

These are rapidly taken up by the tumour cells[60], producing long lasting contrast enhancement of tumour and the remaining nanoparticles are removed from the circulation by reticuloendothelial system[61].

Magnetic microparticle probes with nanoparticle probes have been used for identification of proteins like prostate specific antigen. Here magnetic microparticles coated with antibodies together with nanoprobes with similar coating and a unique hybridized DNA barcode are used. The microparticle coated with antibody directed against prostate specific antigen combines with it to form a complex and can be separated by using magnetic separation. The presence of these separated complexes is determined by dehybridization of the complexed DNA barcode sequence and polymerase chain reaction for the oligonucleotides. This allows prostate specific antigen detection at 30 attomolar concentration[62]. This sensitivity is much greater than conventional assays for prostate specific antigen.

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 these accumulate in their target site by means of an alternating magnetic field applied externally. This heat kills the cancer cells selectively. This method designed by Triton Biosystems, is about to enter clinical trials for solid tumours in 2009[63].

***Nanosomes***

Raoul Kopelman's group at the University of Michigan, USA, has been working on nanosomes also called as PEBBLEs (Probes Encapsulated by Biologically Localized Embedding) which integrate various aspects of medical applications such as targeting, diagnosis and therapy. These nanosomes are being developed for treatment of various tumours, in particular CNS tumours. Silica coated iron oxide nanoparticles coated with polyethylene glycol[64] and affixed with targeting antibody and contrast elements like gadolinium are used to access specific areas of brain involved with tumour. Targeting aids in binding the nanoparticle specifically to the tumour cells and the contrast elements helps in better detection with magnetic resonance imaging. Subsequent treatment with laser can destroy the cells loaded with these nanoparticles by the heat generated by iron oxide particles by absorbing the infra red light. Nanosomes can also be integrated with a photocatalyst which produces reactive oxygen species when stimulated by light and destroy the target tissue. This method has advantage over conventional drugs in being much safer without the adverse effects of cancer chemotherapy drugs and also the absence of development of drug resistance. Nanosomes are being developed to integrate more and more components in it for flexibility of its applications[19,65].

***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 polymerisation[19]. 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[66]. 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[67].

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*[68] have demonstrated the potential use of transferring conjugated PEG modified polyamidoamine (PAMAM) dendrimers for targeted gene delivery to the brain. Pan *et al*[69] have demonstrated the efficacy of magnetic nanoparticle modified PAMAM dendrimers in transfer of antisense *survivin* oligonucleotides in tumour cell lines. These methods provide an effective alternative to viral vectors of gene transfer for treatment of various tumours[69]. 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[70,71].

Dendrimer based drugs are being tried for anti-retroviral therapy[70] and it is in stages of clinical trial after getting clearance from US-FDA on July 2003. This molecule was found to successfully prevent simian HIV infection[19]. PAMAM dendrimers can also be used

in treatment of cancer by conjugating with anti-cancer drugs like cisplatin, adriamycin or methotrexate[72]. Calabretta *et al*[73] have demonstrated the antibacterial property of amino-terminated polyamidoamine (PAMAM) dendrimers and their partially polyethylene glycol (PEG)-coated derivatives. The antibacterial property was observed against both Gram negative *Pseudomonas* and Gram positive *Staphylococcus aureus*. However, the PEG coating of the dendrimer, which reduces the cytotoxicity of unmodified PAMAM dendrimers, reduces the efficacy against Gram positive bacteria without change in efficacy against Gram negative bacteria like pseudomonas[73].

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[74]. Smaller sized dendrimers undergo rapid renal clearance whereas dendrimers with charged surface or hydrophobic surfaces are rapidly cleared by the liver. Those dendrimers with a hydrophilic surface escape renal clearance and have a greater circulation time[75].

Cationic dendrimers have a greater potential to cause cytotoxicity compared to anionic dendrimer or PAMAM dendrimers. It is proposed to cause cell membrane instability and cell lysis. The toxicity of dendrimer is dependent on the size of the particle and increase with size. It can be reduced by means of surface modification of the dendrimers with incorporation of PEG or fatty acids[76].

**Nanotechnology in gene therapy**

Gene therapy is a newer modality of approach for treatment of many genetic disorders including diabetes mellitus[77], cystic fibrosis[78], and alpha 1 antitrypsin deficiency[79]. 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, naked DNA cannot cross the negatively charged cell membrane as these are also negatively charged[79,80]. Hence, there is a need for other modes of transfer of genetic material such as nanoparticle based gene therapy. Liposomes measuring less than 100 nm can be used for delivery of genetic material into cells. Liposomes incorporated with polyethylene glycol and galactose target liver cells effectively due to their rapid uptake by liver Kupffer cells. Thus gene therapy may be tried with such liposomal nanoparticles for various liver disorders such as Wilson's disease and hereditary hemochromatosis[79].

Niu *et al*[77] used human insulin gene in chitosan nanoparticles to transfect STZ diabetic rat through gastrointestinal tract. They found a significant decrease in fasting blood glucose level, increase in plasma insulin levels and expression of human insulin gene mRNA in the study rats. This study may lead to the development of a newer modality of therapy for type 1 diabetes mellitus[77].

A "hybrid nanodevice" designed by workers of Northwestern University and Argonne National Laboratory consists of a titanium dioxide ($TiO_2$) semiconductor nanoparticle of dimension 4.5 nm linked covalently to oligonucleotide DNA[19]. The oligonucleotide sequence can direct the hybrid nanodevice to the corresponding DNA segment within the cell nucleus. When stimulated with light or X rays, $TiO_2$ induces nucleic acid endonuclease which cleaves off the specific DNA fragment. This device is yet to be tested in a laboratory model. However, it may have a potential utility in treatment of various malignancies in the future.

***Respirocytes***

Respirocytes are hypothetical artificial red blood cells[51] are nanodevices which can function as red blood cells but with greater efficacy. These have higher capacity to deliver oxygen to tissues, supplying 236 times more oxygen per unit volume than natural red blood cells. These devices have sensors on the surface which can detect changes in the environment and the onboard nanocomputer will regulate the intake and output of the oxygen and carbon dioxide molecules. An infusion of one litre dose of 50 per cent respirocytes saline suspension in a human can theoretically keep the patient oxygenated up to four hours following cardiac arrest[19,81]. Respirocytes, considered as a device by FDA are regulated under the provisions of the Medical Device Amendments of 1976, the Safe Medical Devices Act of 1990, and the Medical Device Amendments of 1992[82].

***Microbivores***

Microbivores[83] are hypothetical structures which function as white blood cells in the blood stream designed to trap circulating microbes. They are expected to have greater efficacy than cellular blood cells in

phagocytosis. The microbivores surface is arranged with processes which can extend in length and secure the microbe which gets in contact with it. The microbe will be gradually manoeuvred to the ingestion port and undergoes the process of morcellization and enzymatic degradation. The end products are released as amino acids, fatty acids, nucleotides and sugars. Application of the microbivores in human circulation could theoretically clear the blood stream in septicaemia at a much greater rate than the natural defence mechanism with antibiotics[19,83].

**Regulatory challenges with nanomedicines**

Regulatory issues play a major role in the development of nanoformulation drugs. These include, the type of nanodrug produced and the various regulatory requirements that the manufacturers must follow during the manufacturing of nanodrugs. 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[84].

Nanodrug manufacturers must comply with FDA's Current Good Manufacturing Practices (CGMP) and Quality System Regulations (QSR)[85]. Non compliance with these regulations would warrant enforcement actions by the FDA. For example, 21 CFR 211.25 of FDA's CGMP requires that the personnel involved in the manufacturing process, in providing training for the workers and the personnel supervising the manufacturing process have adequate training, education and experience. Further, the training must be conducted at adequate frequency and there must be adequate number of qualified personnel for the assigned duties[86]. A nanodrug manufacturer must invest considerable amount of financial resources to have such qualified personnel in the working unit. Maintenance of equipment for manufacture of nanodrugs and control of contamination are also regulatory requirements for manufacturers. The drug products are purified by the use of filters and CGMP demands that the filters do not release fibres[87]. However, when liquid filtration is used, nanodrug manufacturers will not be able to comply with CGMP, since the smallest filtration level available is approximately 15 nm and nanodrugs could be at the range of 5 to 6 nm long[84].

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 nanomedical 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.[88]

**Potential hazards of nanoparticles**

Nanoparticles, as a result of their extreme microscopic dimension, which gives unique advantage, have potential hazards similar to particulate matter[89]. These particles have the potential to cause varied pathologies of respiratory, cardiovascular and gastrointestinal system[1]. Intratracheal instillation of carbon nanotube particles in mice, has shown that carbon nanotubes have the potential to cause varied lung pathologies like epitheloid granuloma, interstitial inflammation, peribronchial inflammation and necrosis of lung. The toxicity produced by carbon nanotube was found to be greater than that produced by carbon black and quartz[90].

Nanoparticles can enter the central nervous system either directly through axons of olfactory pathway or through systemic circulation that C60 fullerene can cause oxidative stress and depletion of GSH in brain in fishes by entering through the olfactory bulb[91]. Involvement of olfactory bulb in humans is possible in case of inhalational exposure. Studies done on monkeys and rats have shown accumulation of carbon and manganese nanoparticles in the olfactory bulb through the olfactory pathway[92,93]. This shows that nanoparticle mediated delivery can in future provide a means of alternate route, circumventing the blood brain barrier. However, this can also result in the inflammatory reactions in the brain which needs to be evaluated.

Radomski *et al*[94] 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 nanoparticle based drug delivery systems, fullerenes may be a safer approach as compared to nanotubes[1,94].

The toxicity of nanoparticles can also be extrapolated to gastrointestinal system, resulting in inflammatory bowel diseases. The toxicity of nanoparticles may be related to its ability to induce release of pro-inflammatory mediators resulting in inflammatory response and organ damage. If ingested, the nanoparticles can reach the circulation and reach different organs and systems and possibly result in toxicity[95]. These have been studied *in vitro* and in animal models and the effect on human system is difficult to extrapolate from such studies. Their use in humans require further research and much needed caution.

## Conclusion

Although the expectations from nanotechnology in medicine are high and the potential benefits are endlessly enlisted, the safety of nanomedicine is not yet fully defined. Use of nanotechnology in medical therapeutics needs adequate evaluation of its risk and safety factors. However, it is possible that nanomedicine in future would play a crucial role in treatment of human diseases and also in enhancement of normal human physiology. 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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*Reprint requests*: Dr C. Adithan, Director Professor & Head, Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), Dhanvantari Nagar, Puducherry 605 006, India
e-mail: adithan50@gmail.com