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# Biomedical evaluation of nanomedicines

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**Abstract** Nanotechnology is a newly fashionable field but in the world of drug development it is certainly not new. Nanotechnology has a vital role to play in realizing cost-effective diagnostic, therapeutic and prevent tools. The applications of nanotechnology for treatment, diagnosis, monitoring and control of biological systems have recently been referred to as nanomedicine. The nanocarriers have been made of safe materials, including synthetic biodegradable polymers, lipids and polysaccharides. Nanomedicines can be developed either as drug delivery systems or biologically active drug products. Application of nanotechnology is just started in traditional Chinese medicines. The change in the physicochemical and structural properties of engineered nanosized materials with a decrease in size could be responsible for a number of material interactions that could lead to toxicological effects. At present, scientists must accept that it is still very early in the toxicological evaluation for nanomaterials and nanomedicines, and few data on the safety and toxicity. The safety evaluation of nanomedicines includes workforce exposure limits in manufacturing process, environment impact with general impact and to patients after administration and safety for human use, such as depends on route of administration, dose and dosing frequency, as well as safety in drug delivery relates to toxicity of drug payload. The biomedical evaluation of nanomedicines includes biodistribution, metabolic fate, Persistance of non-degradable systems, Specific therapeutic issues and immunogenicity. We hope that the review would attend on the relative issues of nanomedicines with human health and safety and toxicity to develop the evaluation methods of nanoproducts and make nanotechnology play a great role in the progress in nanotechnology and medicines and medicine engineering.

**Key Words** Nanomedicine; nanopharmaceutical; evaluation; nanotechnology; safety; toxicology; bioactivity; biodistribution; biocompatibility; metabolic fate; immunogenicity

## Introduction

Genomics and proteomics research is already rapidly elucidating the molecular basis of many diseases. This brought new opportunities to develop powerful diagnostic, therapeutic and prevent tools. In the future, point-of-care diagnostics will be routinely used to identify those patients requiring preventative medication, to select the appropriate medication for individual patients, and to monitor response to treatment. Nanotechnology has a vital role to play in realizing cost-effective diagnostic, therapeutic and prevent tools. The applications of nanotechnology for treatment, diagnosis, monitoring and control of biological systems have recently been referred to as “nanomedicine” by the National Institutes of Health, USA. Research into the rational delivery and targeting of pharmaceutical, therapeutic and diagnostic agents is at the forefront of projects in nanomedicine. Nanomedicines is a large subject area and includes nanoparticles that act as biological mimetics, nanomachines, nanofibres and polymeric

nanoconstructs as biomaterials, and nanoscale devices sensors and laboratory diagnostics.[1]

"Nanotechnology" is a newly fashionable field but in the world of drug development it is certainly not new. The first nanomedicines are already bringing clinical benefit to thousands of patients. New drugs and new delivery systems both come under the "nanomedicine" umbrella. Drug delivery systems are needed to exploit many of the drugs developed from advances in molecular biology. The challenge is to design innovative devices and technologies able to guide the therapeutic to its correct location of action and ensure that pharmacological activity is maintained for an adequate duration once there. Looking to the future, nanomedicine research is expected to bring significant advances in the diagnosis and treatment of disease. This is still just the beginning. In the longer term, nanomedicines research will certainly embrace the opportunities arising from stem cell research, tissue engineering research and device miniaturisation. Real opportunities exist to design nano-sized bioresponsive systems able to diagnose and then deliver drugs (so-called theranostics), and to design systems able to promote tissue regeneration and repair (in disease, trauma, and during ageing) without the need for chemotherapy. These ideas may today seem science fiction, but to dismiss them too readily would be foolish. The risks and benefits must be carefully addressed to yield useful and safe technologies, but it has been accomplished before, and will be again.[2]

## Medical Applications of nanomedicines

Nanotechnology manifests itself in a wide range of materials that can be useful to medical application. Virtually all of these materials have been designed with chemically modifiable surfaces to attach a variety of legends that can turn these nanomaterials into biosensors, molecular-scale fluorescent tags, imaging agents, targeted molecular delivery vehicles, and other useful biological tools (Fig 1). The unprecedented freedom to design and modify nanomaterials to target cells, chaperone drugs, image biomolecular processes, sense and signal molecular responses to therapeutic agents, and guide surgical procedures is the fundamental capability offered by nanotechnology, which promises to impact drug development, medical diagnostics, and clinical applications profoundly (Fig 2).[3]

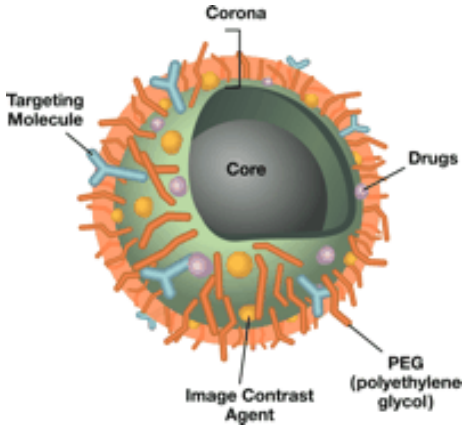


Fig 1. Multifunctional nanoparticle. The nanoparticle's "corona" can be functionalized with hydrophilic polymers, targeting molecules, therapeutic drugs, and image contrast agents. The interior core can be solid (e.g., quantum dots) or liquid (e.g., liposomes). Molecules are not shown to scale. PEG, Polyethylene glycol.( From reference 3)

Nanopharmaceuticals or "Nanomedicines" can be developed either as drug delivery systems or biologically active drug products. They comprise nanometre size scale complex systems, consisting of at least two components, one of which being the active ingredient. Drug delivery is an interdisciplinary area of research that aims at making the administration of complex new drugs feasible, as well as adding critical value to the drugs that are currently in the market. At present, one of the most attractive areas of research in drug delivery is the design of nanomedicines consisting of nanosystems that are able to deliver drugs to the right place, at appropriate times. The goal of the present article is to review the advances in the development and characterization of nanosystems intended to be used as drug carriers for mucosal administration. These nanocarriers are able to protect the associated drug against degradation and facilitate its transport across critical and specific barriers. Some are further able to release the associated drug to the target tissue in a controlled manner. These nanocarriers have been made of safe materials, including synthetic biodegradable polymers, lipids and polysaccharides. A number of nanotechnologies have been developed that enable the association of a variety of drugs to

these nanocarriers, ranging from classical small drug to large DNA fragments. The *in vitro* cell culture studies and the *in vivo* experiments have evidenced the potential of these nanocarriers for overcoming important mucosal barriers, such as the intestinal, nasal and ocular barriers. Hopefully, this will soon represent a strategy for making cheaper and faster, more efficacious medicines.

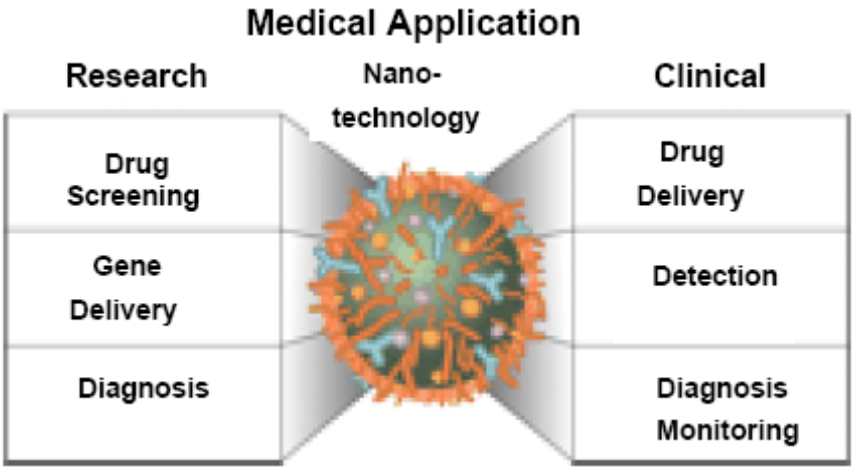


Fig 2. Medical applications of nanotechnology. The size and tailorability of nanoparticles may lea to their widespread use in a variety of medical applications (From Reference 3)

**Pegfilgrastim**

Attaching polyethylene glycol (PEG) to filgrastim avoids the need for the product to be injected daily, according to Graham Molineux, from the Department of Hematology at Amgen, California. Filgrastim is used to avoid febrile neutropenia in patients undergoing bone marrow transplantation. The hypothesis behind the development of pegfilgrastim was that if renal clearance of filgrastim could be eliminated, the only remaining route of removal would be neutrophil-mediated clearance. Neutrophils are actually the product of filgrastim action, so the clearance of the drug could be linked to the drug's effectiveness — the faster the recovery, the quicker the drug is cleared, and the slower the patient's response, the longer the drug persists. Filgrastim has five potential sites for linkage of PEG (via free amine groups on lysine or methionine moieties). In the process of developing the final product more than 40 different pegylated filgrastim molecules were synthesised, using linear and branched chain PEGs, PEGs of different molecular weights and with PEGs of different sizes at different sites. The product that was finally selected has a linear PEG attached to the N-terminal methionine of granulocyte-colony stimulating factor, so that the bulky PEG component does not interfere with the rest of the molecule or its ability to interact with its cognate receptor. Turning to the clinical use of pegfilgrastim, Dr Molineux described how a daily injection of filgrastim enhances the diurnal variation of neutrophil levels. However, pegfilgrastim produces a relentless increase in neutrophil levels. Normal filgrastim is associated with a 48-hour response as it steadily leaks out via the kidneys, but this is not the case with pegfilgrastim. Moreover, the half-life of pegfilgrastim increases with increasing dose, suggesting that there is a saturable element to its clearance. Had a form been developed that was not sensitive to neutrophil-mediated destruction it was possible the drug could have lasted an inordinate length of time in the body, raising concerns about the possibility of excessive exposure to the drug. Phase III studies have been conducted in breast cancer, one trial dosed by weight and the other using a fixed dose. A curious feature of pegfilgrastim is that as body mass increases the bioavailability of the drug increases, with the result that heavy patients are effectively exposed to larger doses. Further studies have shown that the dose effect holds over a wide

range of body weights, although there is still uncertainty about the correct dose for children.

**Polyglutamated paclitaxel**

A polyglutamated form of paclitaxel (Xyotax) has made it possible to deliver large doses of the drug without the need for extensive premedication and without the risk of alopecia, Jack Singer from Cell Therapeutics Inc, Seattle, Washington, told delegates. Conventional paclitaxel injection is formulated in "toxic solubilising agents" because of its poor aqueous solubility. It is given by injection over a three-hour period, preceded by a battery of premedication. Xyotax, however, is water-soluble and can be given over 10 minutes. It achieves plasma levels that are 12 times higher than the conventional form with little or no free drug in the plasma. The absence of free drug in the plasma is thought to account for the low systemic toxicity of Xyotax. Experiments suggest that Xyotax is taken into tumour cells by a process of active endocytosis and that the drug is released as a result of intracellular enzyme action.

Xyotax is a polymer-drug conjugate designed to improve upon the therapeutic index and tolerability of conventional taxanes. Because standard chemotherapy drugs distribute randomly in the body, both tumor and normal tissues are exposed to the cytotoxic effects of these drugs. In contrast, conjugation of low molecular weight drugs, such as paclitaxel, to a polymer results in significant passive tumor targeting by the enhanced permeability and retention effect. Moreover, appropriately designed polymer-drug conjugates form polymeric prodrugs that are inert during transport. The release of the active drug is dependent on cleavage of the polymer backbone following endocytic uptake of the conjugate into tumor cells. Preclinical studies show that CT-2103 accumulates in the tumor tissue and that paclitaxel is slowly and progressively released from the polymer. Clinical pharmacokinetics data show that CT-2103 is stable in plasma; data are consistent with prolonged tumor exposure and reduced systemic exposure to active drug. Based on the promising results in phase I/II studies, 3 phase III trials of CT-2103 were initiated in advanced non-small-cell lung cancer (NSCLC). These Selective Targeting for Efficacy in Lung Cancer, Lower Adverse Reaction (STELLAR) trials represent the largest randomized phase III programs in patients with NSCLC and a poor performance status.[4] Pharmacokinetic investigations indicated a prolonged half-life of >100 hours for conjugated taxanes. Plasma concentrations of unconjugated paclitaxel were similar to those following administration of an equivalent dose of Taxol. Two partial responses were observed, one in a patient with mesothelioma at 177 $mg \cdot m^{-2}$ in phase Ia and one in a patient with gastric carcinoma at 175 $mg \cdot m^{-2}$ in phase I PPX is a water-soluble paclitaxel-polymer conjugate with a prolonged half-life and limited volume of distribution. Dose-limiting toxicities were neutropenia and neuropathy. PPX showed activity in this patient population.[5]

This might form the basis of its ability to overcome the effects of the multi-drug resistance pump, unlike conventional paclitaxel. In addition, Xyotax is synergistic with other cancer treatments. In particular, it increases radio-curability of animal model tumours.

**Stealth liposomal doxorubicin**

There can be 10,000 to 15,000 drug molecules inside a single stealth liposome making the concentration so high that the substance is gelified. Once the liposomes reach tumour tissue, they leak into the interstitial fluid and release their drug cargo. The drug can then diffuse into the tumour cells. Higher concentrations of doxorubicin are therefore achieved in tumour tissue after administration of Doxil than after free doxorubicin and the inhibition of tumour growth is correspondingly greater. Doxil is four times more effective than the equivalent dose of free doxorubicin and a small increase in the dose results in a large increase in the dose delivered to tumour tissue. One critical parameter that affects drug delivery is the diameter of the liposome — particles greater than 400 nm in diameter are hardly extravasated at all and therefore deliver little of the drug. Stealth liposomes that contain other drugs for cancer treatment are being developed, said Professor Gabizon. They include cisplatin, a mitomicin prodrug and a targeted form of Doxil. Stealth liposomes differ from conventional liposomes in that they are coated with polyethylene glycol (PEG). This enhances their hydrophilicity, and enables them to "evade" the

reticulo-endothelial system, thereby slowing their clearance from the body. They have been used to develop "stealth liposomal doxorubicin" (Doxil). Improved efficacy of Doxil (STEALTH liposomal doxorubicin) compared to free doxorubicin has been demonstrated in the treatment of several tumor types. We have shown that addition of low-dose tumor necrosis factor (TNF) to systemic Doxil administration dramatically improved tumor response in the highly vascularized rat soft tissue sarcoma BN175. Whether a similar enhanced efficacy can be achieved in less vascularized tumors is uncertain. We therefore examined the effect of systemic administration of Doxil in combination with low-dose TNF in intermediate vascularized osteosarcoma-bearing rats (ROS-1). Small fragments of the osteosarcoma were implanted s.c. in the lower limb. Treatment was started when the tumors reached an average diameter of 1 cm. Rats were treated with five intravenous injections at 4-day intervals with Doxil or doxorubicin and TNF. Systemic treatment with Doxil resulted in a better tumor growth delay than free doxorubicin, but with progressive diseases in all animals. The 3.5-fold augmented accumulation of Doxil compared to free doxorubicin presumably explains the enhanced tumor regression. Addition of low-dose TNF augmented the anti-tumor activity of Doxil, although no increased drug uptake was found compared to Doxil alone. In vitro studies showed that ROS-1 is sensitive to TNF, but systemic treatment with TNF alone did not result in a tumor growth delay. Furthermore, we demonstrated that treatment with Doxil alone or with TNF resulted in massive coagulative necrosis of tumor tissue. In conclusion, combination therapy of Doxil and low-dose TNF seems attractive for the treatment of highly vascularized tumors, but also of intermediate vascularized tumors like the osteosarcoma.[6] Treatment of cancer through co-administration of anticancer drugs and multidrug resistance (MDR) modulators as a strategy to overcome drug resistance has been extensively explored. However, success has been limited by pharmacokinetic interactions because of non-specific blockade of P-glycoprotein (P-gp) in normal tissues or inability to reach relevant concentrations clinically. Researchers hypothesized that stealth liposomal co-encapsulation of doxorubicin (DOX) with a P-glycoprotein inhibitor, verapamil (DARSLs), may overcome these limitations. Using intravenous administrations, the effects of verapamil (VER) either free (FV) or liposome co-encapsulated with DOX (DARSLs) on the pharmacokinetics and tissue distribution characteristics of DOX either as free (FD) or liposome-encapsulated (LD) were evaluated in normal rats. FV increased ($P$<0.05) the plasma AUC of free DOX (FD). Preparations containing LD had significant prolonged systemic exposure and slow tissue distribution of DOX. LDFV (liposomal DOX with free verapamil) and DARSLs shared similar DOX pharmacokinetics but the latter showed slower DOX distribution in most tissues studied and slower ($P$<0.05) DOX biliary transport. The addition of VER into LD in these two preparations significantly increased the AUC (P<0.01) and reduced the clearance (P<0.01) of DOX when compared to LD. Specifically, DARSLs reduced initial DOX distribution to the heart (P<0.05) corresponding to initial alleviation (P<0.05) of bradycardia when compared to other DOX with VER preparations. In conclusion, liposomal co-encapsulation of DOX with VER has promise of significant therapeutic advantages, and should be explored further in therapeutic studies with animal tumor xenograft models.[7]

**Using magnetic field to deliver nanomedicines**

Nanoparticle-based drug-delivery concept in which an applied magnetic field directs the accumulation in tumor cells of custom-designed, drug-filled nanocarriers has been demonstrated by UB researchers. The new approach, recently published in Molecular Pharmaceutics, may lead to treatments that exploit the advantages of photodynamic therapy (PDT) and that have the potential to reduce drug accumulation in normal tissues. The in vitro results showed that magnetically guided delivery to tumor cells of these customized nanocarriers allowed for more precise targeting, while boosting cellular uptake of the PDT drugs contained inside them.

Once the magnetic field was applied, the concentration of drug inside the tumor cells in the target area increased. Using magnetophoretic control can be to deliver PDT drug to tumor cells, resulting in increased accumulation inside those cells.

Photodynamic therapy is one of the most promising treatments for cancer; it is also being investigated as a treatment method for cardiovascular, dermatological and ophthalmic diseases. The magnetically guided drug delivery would allow for the use of lower concentrations of the drug to deliver a therapeutic dose, thus significantly reducing the amount of PDT drug that accumulates in normal tissue. Cinteza et al reported the design, synthesis using nanochemistry, and characterization of a novel multifunctional polymeric micelle-based nanocarrier system, which demonstrates combined function of magnetophoretically guided drug delivery together with light-activated photodynamic therapy. Specifically, the nanocarrier consists of polymeric micelles of diacylphospholipid-poly(ethylene glycol) (PE-PEG) coloaded with the photosensitizer drug 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a (HPPH), and magnetic Fe3O4 nanoparticles. The nanocarrier shows excellent stability and activity over several weeks. The physicochemical characterizations have been carried out by transmission electron micrography and optical spectroscopy. An efficient cellular uptake has been confirmed with confocal laser scanning microscopy. The loading efficiency of HPPH is practically unaffected upon coloading with the magnetic nanoparticles, and its phototoxicity is retained. The magnetic response of the nanocarriers was demonstrated by their magnetically directed delivery to tumor cells in vitro. The magnetophoretic control on the cellular uptake provides enhanced imaging and phototoxicity. These multifunctional nanocarriers demonstrate the exciting prospect offered by nanochemistry for targeting photodynamic therapy.[8]

Nanocarriers were shown to be efficiently taken up by cultured tumor cells in the area exposed to the magnetic field, as demonstrated by confocal microscopy. A novel nanocarrier system developed from polymer micelles, which are nanosized, water-dispersible clusters of polymeric molecules. Polymeric micelles are excellent nanocarriers for PDT drugs, which are mostly water-insoluble. Along with the photodynamic drug, the UB researchers encapsulated inside the nanocarriers iron oxide nanoparticles, which allowed them to respond to externally applied magnetic fields. While the team has demonstrated this concept with PDT drugs, Prasad said the technique would be useful in delivering gene therapy, chemotherapy or practically any kind of pharmaceutical treatment into cells.

**Nucleoside analogues**

Nucleoside analogues display significant anticancer or antiviral activity by interfering with DNA synthesis. However, there are some serious restrictions to their use, including their rapid metabolism and the induction of resistance. We have discovered that the linkage of nucleoside analogues to squalene leads to amphiphilic molecules that self-organize in water as nanoassemblies of 100-300 nm, irrespective of the nucleoside analogue used. The squalenoyl gemcitabine exhibited superior anticancer activity in vitro in human cancer cells and gemcitabine-resistant murine leukemia cells, and in vivo in experimental leukemia both after intravenous and oral administration. The *squalenoylation* of other antiretroviral nucleosides also led to more potent drugs when tested in primary cultures of HIV-infected lymphocytes. Thus, the squalenoylation is an original technology platform for generating more potent anticancer and antiviral nanomedicines.[9]

Various biomedical applications of carbon nanotubes have been proposed in the last few years leading to the emergence of a new field in diagnostics and therapeutics. Most of these applications will involve the administration or implantation of carbon nanotubes and their matrices into patients. The toxicological and pharmacological profile of such carbon nanotube systems developed as nanomedicines will have to be determined prior to any clinical studies undertaken. This review brings together all the toxicological and pharmacological in vivo studies that have been carried out using carbon nanotubes, to offer the first summary of the state-of-the-art in the pharmaceutical development of carbon nanotubes on the road to becoming viable and effective nanomedicines.[10]

**Nanosystems of drug delivery**

Drug delivery is an interdisciplinary area of research that aims at making the administration of complex new drugs feasible, as well as adding critical value to the drugs that are currently in the market. At present, one of the most attractive areas of research in

drug delivery is the design of nanomedicines consisting of nanosystems that are able to deliver drugs to the right place, at appropriate times. The goal of the present article is to review the advances we have made in the development and characterization of nanosystems intended to be used as drug carriers for mucosal administration. These nanocarriers are able to protect the associated drug against degradation and facilitate its transport across critical and specific barriers. Some of them are further able to release the associated drug to the target tissue in a controlled manner. These nanocarriers have been made of safe materials, including synthetic biodegradable polymers, lipids and polysaccharides. A number of nanotechnologies have been developed that enable the association of a variety of drugs to these nanocarriers, ranging from classical small drug to large DNA fragments. The in vitro cell culture studies and the in vivo experiments have evidenced the potential of these nanocarriers for overcoming important mucosal barriers, such as the intestinal, nasal and ocular barriers. Hopefully, this will soon represent a strategy for making cheaper and faster, more efficacious medicines.[11]

Marriage of cell biology (the concept of "lysosomotropic drug delivery") and the realization that water-soluble synthetic polymers might provide an ideal platform for targeted drug delivery led to the first synthetic polymer-drug conjugates that entered clinical trials as anticancer agents. Conceptually, polymer conjugates share many features with other macromolecular drugs, but they have the added advantage of the versatility of synthetic chemistry that allows tailoring of molecular mass and addition of biomimetic features. Conjugate characteristics must be optimized carefully to ensure that the polymeric carrier is biocompatible and that the polymer molecular mass enables tumour-selective targeting followed by endocytic internalization. The polymer-drug linker must be stable in transit, but be degraded at an optimal rate intracellularly to liberate active drug. Our early studies designed two HPMA [N-(2-hydroxypropyl)methacrylamide] copolymer conjugates containing doxorubicin that became the first synthetic polymer-drug conjugates to be tested in phase I/II clinical trials. Since, a further four HPMA copolymer-anticancer drug conjugates (most recently polymer platinates) and the first polymer-based gamma-camera imaging agents followed. Polymer-drug linkers cleaved by lysosomal thiol-dependent proteases and the reduced pH of endosomes and lysosomes have been used widely to facilitate drug liberation. It is becoming clear that inappropriate trafficking and/or malfunction of enzymatic activation can lead to new mechanisms of clinical resistance. Recent studies have described HPMA copolymer conjugates carrying a combination of both endocrine and chemotherapy that are markedly more active than individual conjugates carrying a single drug. Moreover, current research is investigating novel dendritic polymer architectures and novel biodegradable polymers as drug carriers that will provide improved drug delivery and imaging probes in the future. The present paper reviews the clinical status of polymeric anticancer agents, the rationale for the design of polymer therapeutics and discusses the benefits and challenges of lysosomotropic delivery.[12]

Improving the therapeutic index of medicines is a goal of drug delivery. Employing nanosystems that control drug biodistribution is one way of achieving therapeutic improvements, and polymeric bilayer vesicles are one such nanosystem. Polymeric vesicles, with the ability to transport drugs or genes, are prepared in one of two ways: (i) the self-assembly of amphiphilic polymers and (ii) the polymerisation of monomers, following self-assembly (polymerised vesicles). There are two types of self-assembling amphiphilic polymers: water-soluble polymers derivatised with hydrophobic pendant groups and amphiphilic block copolymers. Amphiphilic alkenes and alkynes are the main compounds that are used to make polymerised vesicles. This review discusses polymer architecture fundamentals that govern the self-assembly of polymers into vesicles, the fine control on vesicle size that is achievable with polymeric vesicles and the application of the vesicles to drug delivery.[13]

The intricate problems associated with the delivery and various unnecessary in vivo transitions of proteins and drugs needs to be tackled soon to be able to exploit the myriad of putative therapeutics created by the biotechnology boom. Nanomedicine is one of the most promising applications of nanotechnology in the field of medicine. It has been defined as the monitoring, repair, construction and

control of human biological systems at the molecular level using engineered nanodevices and nanostructures. These nanostructured medicines will eventually turn the world of drug delivery upside down. PEGylation (i.e. the attachment of polyethylene glycol to proteins and drugs) is an upcoming methodology for drug development and it has the potential to revolutionise medicine by drastically improving the pharmacokinetic and pharmacodynamic properties of the administered drug. This article provides a total strategy for improving the therapeutic efficacy of various biotechnological products in drug delivery. This article also presents an extensive analysis of most of the PEGylated proteins, peptides and drugs, together with extensive clinical data. Nanomedicines and PEGylation, the latest offshoots of nanotechnology will definitely pave a way in the field of drug delivery where targeted delivery, formulation, in vivo stability and retention are the major challenges.[14]

**Synthetic polymers**

The transfer of polymer-protein conjugates into routine clinical use, and the clinical development of polymer-anticancer-drug conjugates, both as single agents and as components of combination therapy, is establishing polymer therapeutics as one of the first classes of anticancer nanomedicines. There is growing optimism that ever more sophisticated polymer-based vectors will be a significant addition to the armoury currently used for cancer therapy.[15]

Synthetic polymers and nanomaterials display selective phenotypic effects in cells and in the body signal transduction mechanisms involved in inflammation, differentiation, proliferation, and apoptosis. When physically mixed or covalently conjugated with cytotoxic agents, bacterial DNA or antigens, polymers can drastically alter specific genetically controlled responses to these agents. These effects, in part, result from cooperative interactions of polymers and nanomaterials with plasma cell membranes and trafficking of polymers and nanomaterials to intracellular organelles. Cells and whole organism responses to these materials can be phenotype or genotype dependent. In selected cases, polymer agents can bypass limitations to biological responses imposed by the genotype, for example, phenotypic correction of immune response by polyelectrolytes. Overall, these effects are relatively benign as they do not result in cytotoxicity or major toxicities in the body. Collectively, however, these studies support the need for assessing pharmacogenomic effects of polymer materials to maximize clinical outcomes and understand the pharmacological and toxicological effects of polymer formulations of biological agents, i.e. polymer genomics.[16]

The last decade has seen successful clinical application of polymer-protein conjugates (e.g. Oncaspar, Neulasta) and promising results in clinical trials with polymer-anticancer drug conjugates. This, together with the realisation that nanomedicines may play an important future role in cancer diagnosis and treatment, has increased interest in this emerging field. More than 10 anticancer conjugates have now entered clinical development. Phase I/II clinical trials involving N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-doxorubicin (PK1; FCE28068) showed a four- to fivefold reduction in anthracycline-related toxicity, and, despite cumulative doses up to 1680 mg/m$^2$ (doxorubicin equivalent), no cardiotoxicity was observed. Antitumour activity in chemotherapy-resistant/ refractory patients (including breast cancer) was also seen at doxorubicin doses of 80-320 mg/m2, consistent with tumour targeting by the enhanced permeability (EPR) effect. Hints, preclinical and clinical, that polymer anthracycline conjugation can bypass multidrug resistance (MDR) reinforce our hope that polymer drugs will prove useful in improving treatment of endocrine-related cancers. These promising early clinical results open the possibility of using the water-soluble polymers as platforms for delivery of a cocktail of pendant drugs. In particular, we have recently described the first conjugates to combine endocrine therapy and chemotherapy. Their markedly enhanced in vitro activity encourages further development of such novel, polymer-based combination therapies. This review briefly describes the current status of polymer therapeutics as anticancer agents, and discusses the opportunities for design of second-generation, polymer-based combination therapy, including the cocktail of agents that will be needed to treat resistant metastatic cancer. [17]

**Carbon nanotubes**

Various biomedical applications of carbon nanotubes have been proposed in the last few years leading to the emergence of a new field in diagnostics and therapeutics. Most of these applications will involve the administration or implantation of carbon nanotubes and their matrices into patients. The toxicological and pharmacological profile of such carbon nanotube systems developed as nanomedicines will have to be determined prior to any clinical studies undertaken. This review brings together all the toxicological and pharmacological in vivo studies that have been carried out using carbon nanotubes, to offer the first summary of the state-of-the-art in the pharmaceutical development of carbon nanotubes on the road to becoming viable and effective nanomedicines.[18]

**Drug nanosization**

Nanosized materials have been investigated as potential medicines for several decades. Consequently, a great deal of work has been conducted on how to exploit constructs of this size range in a beneficial way. Similarly, a number of the consequences from the use of these materials have already been considered. Nanosized materials do behave differently to low-molecular-weight drugs, the biological properties of nanomaterials being mainly dependent on relevant physiology and anatomy, which are reviewed in this article. Biodistribution, movement of materials through tissues, phagocytosis, opsonization and endocytosis of nanosized materials are all likely to have an impact on potential toxicity. In turn these processes are most likely to depend on the nanoparticle surface. Evidence from the literature is considered which suggests that our understanding of these areas is incomplete, and that biodistribution to specific sites can occur for nanoparticles with particular characteristics. However, our current knowledge does indicate which areas are of concern and deserve further investigation to understand how individual nanoparticles behave and what toxicity may be expected from them.[19]

Cancer stem cells (CSCs), i.e. cancer cells that can self-renew, constitute only a minority of the cells of a tumour, but, because of their ability to initiate and repopulate tumours, failure to control CSCs can potentially lead to tumour re-growth, even though the bulk tumour may have been treated successfully. Nanomedicines improve spatio-temporal control over drug kinetics and distribution, thus opening the prospect of safer and more specific therapies to address the challenges posed by CSCs. In particular, these systems have the potential to facilitate CSC-aware therapy by overcoming resistance to conventional cytotoxic drugs and by targeting novel therapies to the tumour and CSC-marker positive cells. This review examines the implications of the CSC paradigm specifically for the development of nanomedicines, i.e. therapies based on macro-molecules or supramolecular aggregates.[20]

Dendrimers have unique molecular architectures and properties that make them attractive materials for the development of nanomedicines. Key properties such as defined architecture and a high ratio of multivalent surface moieties to molecular volume also make these nanoscaled materials highly interesting for the development of synthetic (non-viral) vectors for therapeutic nucleic acids. Rational development of such vectors requires the link to be made between dendrimer structure and the morphology and physicochemistry of the respective nucleic acid complexes and, furthermore, to the biological performance of these systems at the cellular and systemic level. The review focuses on the current understanding of the role of dendrimers in those aspects of synthetic vector development. Dendrimer-based transfection agents have become routine tools for many molecular and cell biologists but therapeutic delivery of nucleic acids remains a challenge.[21]

Interdisciplinary research at the interface of polymer chemistry and the biomedical sciences has produced the first polymer-based nanomedicines for the diagnosis and treatment of cancer. These water-soluble hybrid constructs, designed for intravenous administration, fall into two main categories: polymer-protein conjugates or polymer-drug conjugates. Polymer conjugation to proteins reduces immunogenicity, prolongs plasma half-life and enhances protein stability. Polymer-drug conjugation promotes tumor targeting through the enhanced permeability and retention (EPR) effect and, at the cellular level following endocytic capture, allows lysosomotropic drug delivery. The successful

clinical application of polymer-protein conjugates (PEGylated enzymes and cytokines) and promising results arising from clinical trials with polymer-bound chemotherapy (e.g. doxorubicin, paclitaxel, camptothecins) has provided a firm foundation for more sophisticated second-generation constructs that deliver the newly emerging target-directed anticancer agents (e.g. modulators of the cell cycle, signal transduction inhibitors and antiangiogenic drugs) in addition to polymer-drug combinations (e.g. endocrine- and chemo-therapy).[22]

## Application of nanotechnology in traditional Chinese medicines

Nanotechnoligy is also a rational strategy to promote the modernization of traditional Chinese medicines by improving the clinical effects of crude drugs, and taking the better use of the natural drug resources. One reason is that nanosization for crude drugs and crude extracts may increase the solubilities in biofluid, and hence acquire the higher bioavailabilities. Nano-carrier entrapped form for traditional Chinese medicine may weaken the stimulation of alimentary tract, and avoid the degradation of active components when exposured to the high acidicity and various enzymes. The other way is to prepare non-GI delivered formulations, such as i.v. injections, after the plant extracts were nanosized or nanocarriered, aiming a delayed or controlled release of active components in vivo.[23,24]

Application of nanotechnology is just started in traditional Chinese medicines. Realgar is a toxic drug with very poor solubility. The nanosization of reaglar observed to improve the inhibition intensity to S-180 carcinoma in mice.[25] A further pharmacokinetic experiment also finded that nanosized realgar had an increased bioavailability and a delayed elimination of its active component, arsenic, in rabbits.[26] The nano-particles of *Coneha haliotidis* has also been successfully prepared using specific ball grinding mil1. The structure and surface state of nano-particles were characterized by physical techniques, including TEM，IR，XPS and EDX measurement. Compared with conventional preparation, there is a peak of serum Zn, Ca and Si content when administered with nano-particles. This experiments indicated that the nano-particles of *Coneha Haliotidis* has higher bioavailability significantly.[27]

Table 1. NM effects as the basis for pathophysiology and toxicity. Effects supported by limited experimental evidence are marked with asterisks; effects supported by limited clinical evidence are marked with daggers

| Experimental NM effects | Possible pathophysiological outcomes |
|---|---|
| ROS generation | Protein, DNA and membrane injury, oxidative stress |
| Oxidative stress | Phase II enzyme induction, inflammation, mitochondrial perturbation |
| Mitochondrial perturbation | Inner membrane damage, permeability transition pore opening, energy failure, apoptosis, apo-necrosis, cytotoxicity |
| Inflammation | Tissue infiltration with inflammatory cells, fibrosis, granulomas, atherogenesis, acute phase protein expression (e.g., C-reactive protein) |
| Uptake by reticulo-endothelial system | Asymptomatic sequestration and storage in liver, spleen, lymph nodes, possible organ enlargement and dysfunction |
| Protein denaturation, degradation | Loss of enzyme activity, auto-antigenicity |
| Nuclear uptake | DNA damage, nucleoprotein clumping, autoantigens |
| Uptake in neuronal tissue | Brain and peripheral nervous system injury |
| Perturbation of phagocytic function, "particle overload," mediator release | Chronic inflammation, fibrosis, granulomas, interference in clearance of infectious agents |
| Endothelial dysfunction, effects on blood clotting | Atherogenesis, thrombosis, stroke, myocardial infarction |
| Generation of neoantigens, breakdown in immune tolerance | Autoimmunity, adjuvant effects |
| Altered cell cycle regulation | Proliferation, cell cycle arrest, senescence |
| DNA damage | Mutagenesis, metaplasia, carcinogenesis |

## Physiological principles for Nanomedicines and nanotoxicology

The change in the physicochemical and structural properties of engineered nanosized materials (NM) with a decrease in size could be responsible for a number of material interactions that could lead to toxicological effects. Several Nanosized material characteristics can culminate in reactive oxygen species (ROS) generation, which is currently the best-developed paradigm for nanoparticle toxicity (Table 1).[28]

Nanosized materials have been investigated as potential medicines for several decades. Consequently, a great deal of work has been conducted on how to exploit constructs of this size range in a beneficial way. Similarly, a number of the consequences from the use of these materials have already been considered. Nanosized materials do behave differently to low-molecular-weight drugs, the biological properties of nanomaterials being mainly dependent on relevant physiology and anatomy, which are reviewed in this article. Biodistribution, movement of materials through tissues, phagocytosis, opsonization and endocytosis of nanosized materials are all likely to have an impact on potential toxicity. In turn these processes are most likely to depend on the nanoparticle surface. Evidence from the literature is considered which suggests that our understanding of these areas is incomplete, and that biodistribution to specific sites can occur for nanoparticles with particular characteristics. However, our current knowledge does indicate which areas are of concern and deserve further investigation to understand how individual nanoparticles behave and what toxicity may be expected from them.[28]

## Evaluation on safety and toxicology of nanomedicines

Nanoscale materials are seeming application in direct interventions to improve public health both through therapeutic strategies and environmental remediation. Recent years have seen the emergence of nano-engineered drug delivery strategies. Approval of abraxane, a nano-formulation of taxol for the treatment of breast cancer, was received by FDA. This protein nano-bead conjugated pharmaceutical has increased water solubility allowing for elimination of the toxicity associated with the solvent vehicle and improved therapeutic index. The benefit of abraxane relies on the nanoscale formulation rather than on the emergent properties of the nanomaterials as a therapeutic modality.[29] Powers et al pointed out that basis nanoparticle characterization techniques are discussed, along with some of the issues and implications associated with measuring nanoparticle properties and their interactions with biological systems. Recommendations regarding how to approach nanomaterial characterization include using proper sampling and measurement techniques, forming multidisciplinary teams, and making measurements as close to the biological action point as possible.[30] The science of toxicology has provided the foundation for understanding and studying the interactions between chemical drugs and biology. While the use of nanomaterials, nanomedicines/ nanopharmaceuticals is rather new in the commercial products, the philosophical basis for performing the toxicological evaluation of these products is not expected to be different form other chemical drugs.

At present, scientists must accept that it is still very early in the toxicological evaluation for nanomaterials, nanomedicines/nanopharmaceuticals, and few data on the safety and toxicity. The basic tenet of study designed to develop a study system of toxic effects of nanomaterials, nanomedicines/ nanopharmaceuticals on biological systems is to understand the physico-chemical characteristics of nanomaterials, nanomedicines. Therefore, the approach to addressing the safety and toxicity of these products will best be conducted via multidisciplinary terms. Many traditional methods and approaches will likely be applicable to study of nanomaterials, nanomedicines/nanopharmaceuticals.

Nanotechnology research and development is directed toward understanding and creating improved materials, devices, and systems that exploit these properties. In a review, Thomas et al reviewed that a limited subset of products that contain nanoscale materials, assess the available data for evaluating the consume exposures and potential hazard associated with these products, and discuss the capacity of US regulatory agencies to address the potential risks associated with these products.[31] Some of the

potential impacts of dermal exposure to nanoscale materials include the following: (1) enhanced amount and depth of penetration of active ingredients in cosmetic into the skin resulting in increased activity, (2) ingredients that are chemically unstable in air and light (as retinal and vitamin E) may be more readily used in topical products following encapsulation in nanoparticles, and (3) and timed release of ingredients may become more feasible in topical products and could allow for improved effectiveness equivalent to current controlled release orally administered drugs.

Nanomedicine is the science and technology of diagnosing, treating and preventing disease and improving human health. It is noted that nanomedicine is built on the science and technology of complex systems of nanometer-scale size, consisting of at least two components, one of which is an active principle, and the whole system leading to a special function related to the diagnosis, treatment or prevention of diseases. There is need to improve the understanding of toxicological implications of nanomedicines in relation to the specific nanoscale properties. Risk-benefit assessment is needed in respect of both acute and chronic effects of nanomedicines in experimental animal models and in patients, specially in relation to target diseases. The risk evaluation is very important at the earliest stage of the discovery and then the development of new nanomedicines

All new chemical and pharmaceutical products are tested before humans are exposed to them, either intentionally or accidentally, in order to evaluate any potential hazard associated with such exposure. To determine the magnitude and target of any toxicity, tests are carried out in animals and, subsequently, in humans. Many of these tests are required by national and international regulatory authorities. New compounds are tested, for specific ethical and regulatory reasons, to identify possible adverse effects and mechanisms of toxicity. It is widely recognized that there is a need to improve the current testing methods to maximize the relevance of the information generated with respect to the prediction of adverse effects in humans. Several *in vivo* animal models have been used to assess the testicular toxicity of many compounds. These models entail the sacrifice of animals and the determination of different enzymes, sperm motility, and testis morphology.[32,33] To avoid testing in several species of animals *in vitro*, systems that provide information on species-specific metabolism, pharmacokinetics, and toxicology are essential.

The safety evaluation of nanomedicines includes workforce exposure limits in manufacturing process, environment impact with general impact and to patients after administration and safety for human use, such as depends on route of administration, dose and dosing frequency, as well as safety in drug delivery relates to toxicity of drug payload.

Table 2. The biomedical evaluation of nanomedicines

| Evaluation terms | Evaluation contents |
|---|---|
| Biodistribution | Whole organism, cellular level |
| Metabolic fate | Absorption, distribution, metabolism and excretion |
| Immunogenicity | IgG/IgM production, cytokine induction |
| Persistance of non-degradable systems | Possibility of lysosomal storage disease |
| Biocompatibility | Biological environment and toxicology and adverse effect to patients |
| Specific therapeutic issues | Therapeutic index of nanomedicines and its delivery systems in drug delivery relates to toxicity pf drug payload |

Due to the nanotechnology combines with biotechnology, a newly emerging cross-disciplinary field nanobiotechnology. This becomes the new developing area. As the research and application of nanotechnology, studying and understanding the complex relationship between nanomaterials/ nanomedicines and biological system will show special important to environmental, human health and safety. Criticism of the use of laboratory animals for the safety testing of chemicals is increasing, in

society as a whole and also in the scientific world. This criticism is not only limited to ethical concerns, but scientific considerations also play a significant role. It should be realized that the animal bioassays presently used in toxicity testing are model systems for the prediction of toxicity in humans or the environment. In the last few decades new technologies and new knowledge have become available. This development is the result of intensive fundamental toxicological research and the implementation of new methods and technologies.[34] The biomedical evaluation of nanomedicines includes biodistribution, metabolic fate, Persistance of non-degradable systems, Specific therapeutic issues and immunogenicity (Table 2).[35]

The toxicology of nanomedicines used in device manufacture should be considered during their entire life cycle at stages of manufacture and preclinical and clinical development, consumer and staff safety and waste management in environment. The development of *in vitro* models of testicular toxicity may provide important tools for investigating specific mechanisms of toxicity in the testis. Although various systems have been reported, their application in toxicological studies has been limited by the poor ability to replicate the complex biochemical, molecular, and functional interactions observed in the testis. *In vitro* models have been established, and some of them have tried to reproduce the complex interactions that take place between the different germ cells. These models are limited by the poor viability of freshly isolated germ cells. So the development of a germ-line stem cell is of great interest. After previous studies to develop an immortalized cell line[36], the authors finally obtained a cell line[37,38] with promising application in the study of testis toxicity. In vivo system for evaluation on safety and toxicology is very importance. This evidence of physiologically significant histopathological changes clearly indicates the potential of these nanomaterials for human toxicity at realistic doses. The Occupational Safety and Health Administration (OSHA) standard described above must be reviewed in light of this accumulating evidence of mammalian pulmonary toxicity.[39]

Cancer therapy has benefited from the use of liposomal doxorubicin, a formulation that again increases the therapeutic index of the active agent through a combination of passive tumor targeting and reduced toxicity.[40] In this case, coating the liposome with PEG significantly decreases uptake by macrophages and allows the liposomes to concentrate in tumors by escaping from the leaky vasculature surrounding solid tumors [41] through a phenomenon known as the enhanced permeation and retention (EPR) effect.[42-44]

## Conclusion

Nanotechnology is a relatively young field, it is developing rapidly, thanks to a strong foundation of material science and engineering. Biologists are using this innovative technology to overcome boundaries common to cell biology and clinical medicine. As more biologists learn about the capability of nanotechnology and develop cross-disciplinary collaborations with physicists, engineers, material scientists, pharmacologists, and medical scientists, these breakthroughs will undoubtedly increase in magnitude and quantity. The applications of nanotechnology for treatment, diagnosis, monitoring and control of biological systems have recently been referred to as nanomedicine. The nanocarriers have been made of safe materials, including synthetic biodegradable polymers, lipids and polysaccharides. Nanomedicines can be developed either as drug delivery systems or biologically active drug products. Application of nanotechnology is just started in traditional Chinese medicines. The change in the physicochemical and structural properties of engineered nanosized materials with a decrease in size could be responsible for a number of material interactions that could lead to toxicological effects. At present, scientists must accept that it is still very early in the toxicological evaluation for nanomaterials and nanomedicines, and few data on the safety and toxicity. The safety evaluation of nanomedicines includes workforce exposure limits in manufacturing process, environment impact with general impact and to patients after administration and safety for human use, such as depends on route of administration, dose and dosing frequency, as well as safety in drug delivery relates to toxicity of drug payload. The biomedical evaluation of nano-medicines includes biodistribution, metabolic fate, Persistance of non-degradable systems, Specific

therapeutic issues and immunogenicity. We hope that the review would attend on the relative issues of nanomedicines with human health and safety and toxicity to develop the evaluation methods of nanoproducts and make nanotechnology play a great role in the progress in nanotechnology and medicines and medicine engineering.

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