

# Nanotechnology: its role in Oncology

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## Abstract

Nanoparticles(NPs) have unique physicochemical properties that are difficult from those of the bulk material. Properties such as small size (< 100nm), relatively high surface-to-volume ratio, quantum dot effect and reactivity, allow a wide range of different applications in modern industry. Nanotechnology is considered as a technology of the future, with a greater potential in biochemical applications. It is becoming increasingly important in nano-diagnostic (medical imaging, biosensors, contrast agents and invitro 'lab-on-a chip'), drug-delivery devices (for gene delivery and targeting drugs) and for treating a variety of human tumors. Researches have developed a novel cancer-targeting nano-particle that can potentially act as a drug-delivery agent and dual imaging agent at the same time. It is well said that

" Successful development of targeted cancer therapeutics is vital, as many current chemotherapy regimens lead to severe systemic toxicity due to the fact that the toxic drugs will kill healthy tissue whilst in circulation....".

Targeted drugs will hopefully reduce adverse reactions by limiting their action to cancer tissue only. Nanoparticles can easily be functionalized to target specific types and may be promising delivery and imaging in the treatment of cancer. The clinical feasibility of nanoparticle therapeutics will depend on the effectiveness, safety and cost. Nanoparticle capable of site-specific and intracellular delivery combined with optimal RNA-design are needed to maximize the therapeutic efficacy to reduce dosage and non-specific effects. " Nano-scientists should.... Focus their efforts on nano-oncology. So far studies carried out suggest they could make a significant impact in treating various malignancies".

## INTRODUCTION

Nanotechnology is the development of engineered device at the atomic, molecular and macromolecular level in nanometer range. Nanoparticles have potential application in medical field including diagnostic and therapeutics.

Nanotechnology devices are being developed for early diagnosis of cancer by generating in-vivo sensors to detect location of tumour and early detection of metastatic changes. Advances in nano-technology also proved beneficial in therapeutic field such as drug-discovery, drug-delivery and gene/protein delivery. Nanoparticles can be constructed by various methodology so that effect can be targeted at the desired site.<sup>1-3</sup>

Nanotechnology is the creation of very small particle device and system. This technology takes place at a very minute level. One nanometer = one-billionth of a meter; the width of about

5 atoms. This technology is being developed and designed to interact with cells at a molecular(sub-cellular) level with high degree of functional specificity, thus allowing integration between the device and biological

system. Nanoscale devices are 100-10000 times smaller than the human cell. Because of their small size and larger surface area relative to their volume, nano-scale devices can readily interact with biomolecules (enzymes, receptors) on both surface of cell and inside the cell.<sup>4-6</sup> The use of materials in nano-scale provides more freedom to modify fundamental properties such as solubility, diffusivity, blood circulation  $t^{1/2}$ , drug release characteristics, and immunogenicity. These nano-scale agents may provide more effective and/or more convenient route of administration, lower therapeutic toxicity, extend the product life-cycle, and ultimately it reduce the health care costs.<sup>4-5</sup>

As therapeutic delivery system, nano-particle allow targeted delivery and controlled release. For diagnostic applications, nano-particles allow detection of the molecular scale: they help identify abnormalities such as fragments of viruses, pre-cancerous cells, and disease markers that cannot be detected with traditional diagnostics. Nano-particle based imaging contrast agents have also been shown to improve the sensitivity and specificity of magnetic resonance imaging.<sup>7-8</sup>

Advantages of nano-particle based drug delivery system.<sup>6-7</sup>

- it improves the solubility of poorly water soluble drugs.
- it prolongs the half-life of drug systemic circulation by reducing immunogenicity.
- it release drug at a sustained rate or in an environmentally responsive manner and thus decreases the frequency of administration.
- delivers drug in a target manner to minimize systemic side-effects.
- it can deliver two- or more drug simultaneously for combination therapy to generate synergistic effect and suppress drug resistance.

## NANO-PARTICLE BASED THERAPEUTICS AND CLINICAL USE

More than 150 companies are developing nano-sale therapeutics. 24 nanotechnology based therapeutic products have been approved for clinical use. Among these products, liposomal drugs and polymer-drug conjugates are two dominant classes, accounting for more than 80% of the total amount.

Liposomes are spherical lipid vesicles with a bilayered membrane structure composed of natural or synthetic amphiphilic lipid molecules. Liposomes have been widely used as pharmaceutical carriers because<sup>9-11</sup>----

- ability to encapsulate both hydrophilic and hydrophilic therapeutic agents with high efficiency.
- can protect the encapsulated drugs from undesired effects of external conditions.
- can be functionalized with specific ligands that can target specific cells, tissues, and organs.
- can be coated with inert and biocompatible polymers such as PEG, thereby prolonging the liposome circulation half-life in-vivo, and
- can form desired formulations with needed composition, size, surface charge, and other properties.

**Figure 1**

Table-1 Clinically Approved Nano-drugs for Use in Oncology

Composition	Indication	Administration route
<b>1—Liposomal formulations</b>		
Cytarabine	Malignant lymphomatous meningitis	IT
Doxorubicin	HIV-related Kaposi sarcoma	IV
Doxorubicin	Combination therapy with cyclophosphamide in metastatic breast carcinoma	IV
PEG-Doxorubicin[polyethyleneglycol-doxorubicin]	HIV-related Kaposi's sarcoma, metastatic breast cancer, metastatic ovarian cancer	IM
<b>2—Polymeric formulations</b>		
Methoxy-PEG-Poly[D,L-Lactide]	Taxol	IV
PEG-Granulocyte-colony stimulating factor[G-CSF]	Neutropenia associated with cancer chemotherapy	Sub-cut
PEG-L-asparaginase	Acute lymphoblastic leukemia	IV / IM
<b>3—Other formulations</b>		
Albumin-bound paclitaxel	Metastatic breast cancer	IV

PEG-Doxorubicin[polyethylene glycol-doxorubicin] ie doxil was the first liposomal drug formulation approved by the USA-FDA for the treatment of AIDS-associated Kaposi sarcoma in 1995. It was done by encapsulating doxorubicin into stealth liposome carriers comprised of hydrogenated soy phosphatidyl choline, cholesterol, and PEGylated phosphoethanolamine.

Liposomal-PEG doxorubicin prolonged doxorubicin circulation  $t^{1/2}$  and enhanced drug disposition in the tumor tissue. Other liposomal drugs used in clinical practice include DepoCyt [cytarabine liposomes]. Another nano-particle drug delivery platform in clinical practice is Polymer-drug conjugates.<sup>12,13,14</sup>

- Small-molecule therapeutic agents have two unfavourable properties
- Short circulation  $t^{1/2}$ , which leads to frequent administration.
- Non-site-specific targeting, resulting in undesired systemic side-effects.

The conjugation of small-molecule drugs to polymeric nano-carriers can improve undesirable adverse effects.

- Polymer-drug Conjugates<sup>10,13</sup>
- Prolong the in-vivo circulation time from several minutes to several hours.
- Reduce the cellular up-take to the endocytic route thereby enhancing the passive delivery of drugs to tissues with leaky blood vessels, such as tumors and atherosclerotic plaques. Many polymers can act as drug delivery carriers.

PEG was 1<sup>st</sup> introduced into clinical use in early 1990s. The advantage of PEG is that it enhances the plasma stability; it

enhances the solubility of drug and reduces immunogenicity. Polymeric drugs acting as drug carrier can be PEG, Polyglutamic acid, polysaccharide. poly[allylamine hydrochloride] and macromolecule –drug conjugate having hydrodynamic size of 5-200nm. An example is abraxane, a 130-nm albumin bound paclitaxel drug, that was approved by the FDA in 2005 as second line treatment for patients with breast cancer. This drug concentrates in the tumor partly through the passive enhanced permeability and retention effect and partly through the transendothelial transport mechanisms via the albumin binding protein gp-60. This drug doubles the therapeutic response rate and also increases time to disease progression and overall survival in patients with breast cancer.

## MAIN DRAW-BACK OF LIPOSOMES

Fast clearance of liposomes from the blood by phagocytic cells of the reticulo-endothelial system (RES), resulting in unfavourable therapeutic index. The strategies to overcome this problem is to formulate long-circulating liposomes by coating the liposome surface with inert and biocompatible polymers such as PEG. The polymer layer provides a protective shell over the liposome surface and suppresses liposome recognition by opsonins, and therefore subsequent recognition by RES.

Another strategy is to increase accumulation of liposomes in desired cells, tissues, and organs. This is done by attaching target ligands such as antibodies, peptides and small molecules (folate and transferrin) to the liposome surface. Target liposomes have been developed for differential drug delivery. eg liposomal formulation of vincristine for the treatment of aggressive NHL. Using liposomal-based transmembrane carrier system [TCS], liposomal vincristine-TCS, has the ability to target intracellularly. Phase-I and II trials on liposomal vincristine have demonstrated that this preparation has longer blood circulation  $t_{1/2}$ , higher accumulation in tumors and more sustained drug release profile than free vincristine. Therefore liposomal vincristine can potentially increase efficacy of vincristine and decrease adverse effects of drug. In addition release of drug in a controlled manner can also increase the therapeutic efficacy. By incorporating a fraction of pH-sensitive phosphatidylethanolamine, dimethyldioctadecylammonium bromide, or oleyl alcohol into liposomal membrane, smart liposomes have been developed for preferential various intracellular drug delivery. These liposomes are generally stable in blood while undergoing phase transition under endosomal pH.<sup>19</sup>

PEG has been widely used to enhance the pharmacokinetics of various nano-particle formulations. PEG is a highly hydrated flexible polymer chain that reduces plasma protein adsorption and biofouling of nano-particles while reducing renal clearance of relatively smaller drug molecules, and thus prolongs drug circulation half-life. PEG is also non-toxic and non-immunogenic, making it suitable for clinical applications. These favourable characteristics have led to many new-pegylated products under various phases of clinical evaluation. e.g PEG-arginine deaminase is in phase-II trial for treating Hepato-cellular carcinoma.

Another polymer employed to formulate polymer drug-conjugates is N-(2-hydroxypropyl)methacrylamide [HPMA]. HPMA is a linear hydrophilic polymer with functionalizable side-chains that can be activated to enable drug attachment or conjugation with target ligands. By conjugating small hydrophobic drugs such as paclitaxel to an HPMA polymer, drug water solubility is highly improved. This makes drug formulation and patient administration easier. In addition, HPMA is biodegradable and non-immunogenic. Due to above facts, a number of HPMA products have been developed and are under clinical trials, eg HPMA-co-polymer-diaminocyclohexane palatinatate in clinical trials phase-II for treating recurrent ovarian cancer. FCE28069 [HPMA co-polymer-doxorubicin-galactosamine, in phase-II for HCC, and PNU166945 (HPMA-co-polymer-paclitaxel) in phase-I to document its toxicity and pharmacokinetics for treating refractory solid-tumors.

Different nano-particle platform can be:

drug-encapsulated liposomes; polymer-drug conjugates; nano-emulsions; dendrimers and inorganic nano-particles.

**Figure 2**

Composition	indication	administration	Status
<b>Liposomal platforms</b>			
L-annamycin	ALL, AML	I.V	Phase-I
L-cis-platin	Progressive osteogenic sarcoma metastatic to the lung	aerosol	Phase-II
L-hurtotecan	Ovarian cancer	I.V	Phase-II
L-vincristine	NHL	I.V	Phase-II/III
<b>Polymeric platforms</b>			
HPMA-polymer-DACH[diaminocyclohexane] palatinatate	Ovarian cancers	I.V	Phase-II
PEG-arginine de-aminase	Hepatocellular carcinoma (HCC)	I.V	Phase-I/II
PEG-camptothecin	Various cancers	I.V	Phase-I/II
Pluronic block-co-polymer doxorubicin	Esophageal cancer	I.V	Phase-II
Polycyclodextrin camptothecin	Metastatic solid tumors	I.V	Phase-I
Polyglutamate-camptothecin-camptothecin	Colo-rectal and ovarian cancers	I.V	Phase-I/II
Polyglutamate-paclitaxel	NSCLC	I.V	Phase-III
Poly[iso-hexyl-cyanoacrylate] doxorubicin	HCC	i.a	Phase I/II
<b>Other Platforms</b>			
Nano-crystalline 2-methoxyestradiol	Various cancers	oral	Phase-II
Paclitaxel nanoparticles in porous, hydrophilic matrix	Solid tumors	i.v	Phase-I

## NANO-PARTICLE BASED THERAPEUTICS

## UNDER CLINICAL TRIALS IN ONCOLOGY

**Figure 3**

Table-3 Nano-particle Based Therapeutics in Pre-clinical Development

Composition	therapeutic	Indication
<b>Polymeric micelle</b>		
Immunoenzymosomes[antibody-enzyme-conjugated nano-particles]	Antibody directed enzyme pro-drug therapy	Ovarian cancer
Biotinylated Ab-conjugated polymeric micelles	Doxorubicin	Brain targeting
Pluronic block co-polymers	Doxorubicin	Various cancers
Polymer lipid hybrid nano-particles	Doxorubicin	Solid tumors
Polymerosomes	Hemoglobin	Oxygen carrier
Poly(lactic-co-glycolic acid)-block-poly(ethylene glycol)	Doxetane	Prostate cancers
Poly(vinyl alcohol)/PVA polymeric micelle	PVA polymer antitumor activity	Neuroblastoma, melanoma
<b>Dendrimers</b>		
Folic acid-PAMAM[poly(amidoamine)] dendrimers	Methotrexate	Epithelial cancers
Ligand-conjugated PEG/poly ethylene glycol-poly-L-Lysine dendrimers	Chloroquine-phosphate	Malaria
Polypropyleneimine dendrimers	Efavirenz	HIV-infection
Poly(glycerol-sebacic acid) dendrimers	Camptothecin	Various cancers
<b>Albumin Based Nano-particles</b>		
Albumin bound nano-particles	Doxorubicin, Methotrexate	Various cancers
Cationic albumin-PEG nanoparticles	NC-1900 Vasopressin fragment analog	Scopolamine –induced memory deficits
<b>Polysaccharide based nanoparticles</b>		
Aerosol OT[AOT]–alginate nanoparticle	Doxorubicin	CA-Breast
Glycol chitosan nanoparticles	Doxorubicin	Solid tumors
<b>Virus based nanoparticles</b>		
Cowpea mosaic virus-PEG NPs	Gene therapy	Various purposes
Gold-conjugated CMV-NPs	Phototherapy, Gene therapy	Solid tumors
<b>Metallic NPs</b>		
Anti-HER2[human epidermal growth factor receptor2] Ab-targeted gold/silicon NP.s	Nanoshell-assisted infra red-photothermal therapy	Metastatic breast-CA
Aminosilane-coated iron oxide NP.s	thermotherapy	Brain tumors
Starch-coated iron oxide NP.s	Magnetically guided mitoxantrone	Tumor angiogenesis
Ceramic NP.s		
Silica based NP.s	Photodynamic therapy	Various cancers
Silica cross-linked block copolymer micelle	Imaging agents, chemotherapies	Imaging, chemotherapy

More complex nano-particle system are under development. These include increasing numbers of nano-scale vehicles with distinct chemical, physical, and biological properties for myriad of clinical indications.

Besides liposomes and polymeric conjugates, other nano-particle platform includes polymeric nano-particles., micelles., nano-shells., dendrimers, engineered viral nanoparticles., albumin-based nano-particles., polysaccharide-based-nano-particle., metallic nano-particles and ceramic nano-particles.<sup>21,23</sup>

Biodegradable polymeric micelles with a size of 10-200nm act as drug delivery nano-carriers with therapeutic potential. Polymeric micelle are formed by self-assembly of block copolymers consisting of two or more polymer chains with different hydrophobicity. These copolymers spontaneously assemble into a core-shell micellar structure in an aqueous environment to minimize the system's free energy.

Specifically, the hydrophobic blocks form the core to minimize their exposure to aqueous surroundings, whereas Hydrophilic blocks form the corona-like shell to stabilize the core through direct contact with water. This micellar structure provides an ideal drug delivery nanocarrier. Its hydrophobic core is capable of carrying pharmaceuticals , especially poorly soluble drugs, with high loading capacity (5-25% weight). Its hydrophilic shell provides a steric protection for micelle, thereby increasing its stability in

blood. It also provides functional groups suitable for further micelle modification.

Each polymeric micelle can carry more drugs due to its considerably large size and can release drug in more regulated manner. The encapsulated drugs can be released through surface or by bulk erosion of the biodegradable polymers or by diffusion of drug through polymer matrix or by polymer swelling followed by drug diffusion.

External conditions such as change of pH and temperature can also trigger drug release from polymeric micelles.

Polymeric micelles are more stable in blood than liposomes and other surfactant micelles because some amphiphilic copolymers have a considerably lower critical micelle concentration value.

These polymeric micelle systems can also be used to co-deliver two or more drugs with similar or different water solubility for combination therapy or to simultaneously deliver two or more therapeutic modalities such as radiation agents and drugs.

The surface modification of these micelles with ligands such as antibodies, peptides, nucleic acid aptamers, carbohydrates and small molecules can differentially target their delivery and uptake by subset of cells, which will further increase their specificity and efficacy and reduce their systemic toxicity.

## BIODEGRADABLE POLYMERS

Poly[D,L-lactic acid], Poly[D,L-glycolic acid], Poly[epsilon-caprolactone] at various molar ratio with PEG are the most commonly used biodegradable polymers to form micelle for drug delivery.

Dendrimers are the novel class of drug delivery-NP platform .Dendrimers are globular, highly branched, and synthetic polymers consisting of an initiator core and multiple layers with active terminal groups. These layers are comprised of repeating units and each layer is called a generation. The core of a dendrimer is denoted as generation zero. The specific molecular structure of dendrimers enable them to carry various drugs using their multivalent surfaces through covalent conjugation or electrostatic adsorption. Alternatively dendrimers can be loaded with drugs using the cavities in their cores through hydrophobic interaction, hydrogen bond, or chemical linkage.

Recently, researchers have developed PAMAM[poly amidoamine-based G5 dendrimer] , which has a diameter of

5nm and more than 100 functional primary amines on surface. Here folate is attached as targeting molecule and methotrexate as therapeutic agent, the G-5 dendrimer was about ten times more effective than methotrexate alone in prohibiting tumor growth.

Also, the targeted methotrexate-loaded dendrimer have less systemic toxicity than free methotrexate. so PAMAM-drug delivery system can optimize drug accumulation in tumor and therapeutic efficacy.

Albumin-, Polysaccharide-, and virus-based NP,s represent another class of NP's platforms comprising of biopolymers. These NP's have peculiar biological characteristics.

If a small molecule drugs are conjugated with human serum albumin or a polysaccharide [such as chitosan] , their stability and biodistribution can be significantly improved.

Viruses can be regarded as living NP's with a core shell structure. The core contains infectious agents that can control transcription and translation machinery of host cells. The shell is comprised of various proteins or proteins embedded in lipid membranes. Virus-based NP's have been used as gene-delivery vehicles due to their high gene transfection efficiency.

Usually soft-nanoparticles but also hard-nanoparticles eg metallic NP's, ceramic NP's are also available.<sup>20,21,23</sup>

Example of metallic-nanoparticle are iron-oxide, which can be used as passive or targeting agent after being coated with dextran, surfactants, phospholipids, to improve their stability. Gold-NP's have good optical and chemical property and thus show high infra-red phototherapy potential. Aminosilane-coated iron-oxide nanoparticle have been utilized in thermotherapy to treat brain tumor in rat model. These can increase survival time 4.5 fold over control. Ceramic nanoparticle such as silica, titania, alumina are bio-inert and have porous structure. These nanoparticles have been proposed as drug delivery vehicle to carry drugs for various cancer therapies.<sup>19-22</sup>

Nano-toxicity and major problems with Nano-particle Delivery are: polymer toxicity; immunogenicity; non-specific bio-distribution; in-vivo circulation instability; low-drug carrying capacity; rapid drug release and manufacturing problems.

## CONCLUSION

More than 20 NP- therapeutics are under clinical use. NP's

can improve therapeutic index of drugs. There are certain approved NP's and some NP's are under pre-clinical and clinical development, including liposomes, polymeric micelles, dendrimers, Q-dots, gold-NP's, and ceramic-NP's. The next generation-nanoparticle's may have targeting ligands such as antibodies, peptides, or aptamers, which may further improve their efficacy or reduce their toxicity.

## References

1. Silva GA. Introduction to nanotechnology and its application to medicine. *Surg Neuro*. 2004; 61: 216.
2. Service RF. Nanotechnology grows up. *Science*. 2004; 304: 1732.
3. Kawasaki ES, Player A. Nanotechnology, nanomedicine, and the development of new, effective therapies for cancer. *Nanomedicine*. 2005; 1: 101-109.
4. Emerich DF, Thanos CG. Targeted nano-particle-based drug delivery and diagnosis. *J Drug Target*. 2007; 15: 163-183.
5. Bhat JS. Heralding a new future-Nanotechnology ? *Curr Sci* 2003; 85:147.
6. Groneberg DA, Giersig M, Welte T. Nanoparticle-based diagnosis and therapy. *Curr Drug targets*. 2006; 7: 643-648.
7. Farokhzad OC, Langer R. Nanomedicine : developing smarter therapeutic and diagnostic modalities. *Adv Drug Deliv Rev*. 2006; 58: 1456-1459.
8. Brannon-peppas L, Blanchette JO. Nanoparticle and targeted system for cancer therapy. *Adv Drug Deliv Rev*. 2004; 56: 1649-1659.
9. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov*. 2005; 4: 145-159.
10. Northfelt DW. Pegylated-liposomal doxorubicin versus doxorubicin, bleomycin, and vincristine in the treatment of AIDS-related Kaposi sarcoma: results of a randomized phase-III clinical trial. *J Clin Oncol*. 1998; 16: 2445-2451.
11. Duncan R. Polymer conjugates as anti-cancer nanomedicines. *Nat Rev Cancer*. 2006; 6: 688-701.
12. Harries M, Ellis P, Harper P. Nanoparticle albumin-bound paclitaxel for metastatic breast cancer. *J Clin Oncol*. 2005; 23: 7768-7771.
13. Woodle MC. Controlling liposome blood clearance by surface grafted polymers. *Adv Drug Deliv Rev*. 1998; 32: 139-152.
14. Sapra P, Allen TM. Internalizing antibodies are necessary for improved therapeutic efficacy of antibody-targeted liposomal drugs. *Cancer Res*. 2002; 62: 7190-7194.
15. Simoes S, Moreira JN, Fonseca C, Duzgunes N, de Lima MC. On the formulation of pH-sensitive liposomes with long circulation times. *Adv Drug Deliv Rev*. 2004; 56: 947-965.
16. Bissett D. Phase-I and pharmacokinetic study of MAG-CPT : a polymeric derivative of camptothecin . *Br J Cancer*. 2004; 91: 50-55.
17. Seiler MP. Dendritic cell function after gene transfer with adenovirus-calcium phosphate co-precipitates. *Mol. Ther*. 2007; 15: 386-392.
18. Kabanov AV, Batrakova EV, Alakhov VY. Pluronic block copolymers as novel polymer therapeutics for drug and gene therapy. *J Control Release*. 2002; 82: 189-212.
19. Torchilin VP. Micellar nanocarriers: pharmaceutical perspectives . *Pharm Res* 2007; 24:1-16.
20. Morgan MT. Dendrimer-encapsulated camptothecins: increased cellular uptake, and cellular retention affords enhanced anti-cancer activity in vitro. *Cancer Res*. 2006; 66: 11913-11921.

21. Chavanpatil MD, Khdair A, Panyam J. Surfactant–polymer nano-particles : a novel platform for sustained and enhanced cellular delivery of water–soluble molecules. *Pharm Res.* 2007; 24: 803-810.
22. Jordan A. The effect of thermotherapy using magnetic nano-particles on rat malignant glioma. *J Neuro oncol.* 2006; 78: 7-14.
23. Jurgons R. Drug loaded magnetic nanoparticles for cancer therapy. *J Phys Condens Matter.* 2006;18: S2893-S2902.

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