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Nanotechnology unbolting new avenues for targeted delivery of cancer therapeutics: A brief overview

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Graphical Abstract

Abstract.

The particles within the nanoregime are quite smaller (100- 10,000 times) than human cells but are comparable to that of biomolecules like enzymes and receptors. The nanoparticles smaller than 50 nm can easily pervade into most cells, and those particles smaller than 20 nm can easily escape into the circulation through the blood vessels. Nanoparticles are quite conducive to be fabricated appropriately to serve as a device/vehicles of important therapeutic genes or drugs specifically to the cancer cells avoiding any hazardous

effects on the normal cells. This review encompasses the recent investigations employing nanocarriers like liposomes, micelles, carbon nanotubes, dendrimers, nanoshells that has been developed with positive results.The cancer therapeutic agents like Doxorubicin, Paclitaxel, Cystatin ,Small interfering RNA(SiRNAs) are encapsulated within these nanocarriers through the processes of entrapping, covalent binding, encapsulation or adsorption. Furthermore these nanoparticles were conjugated with cancer specific targetic ligands like Folic acid, Monoclonal antibody,Luteinising hormone releasing hormone(LHRH)peptide,etc which enable them to successfully deliver the therapeutic agents to the cancerous cells. Henceforth the development of these smart nanodevices will undoubtedly pave the way for coming up with future novel therapeutic strategies for combating the malignant cells circumventing any adverse side effects on the normal cells.

Keywords:Liposomes;Micelles; dendrimers;Carbon nanotubes;

	Targeted delivery;cancer therapeutics.

Introduction

Nanotechnology can exert immense impact on cancer therapy as it directly aids in the delivery of the chemotherapeutic drugs.The therapeutic efficacy of all the commonly used drugs can be augmented considerably through the proper utilization of nanotechnology[**Barett *et al* 2004].**These approaches will help in delivering the drug or therapeutics specifically to the target site without hampering the other cells [**Vasir *et al* 2005,Kipp,2004**].Furthermore those drugs which have previously failed clinical trials could be readministered after combining with appropriate nanotechnological measures [**Rabinow,2004**]. Nano drug delivery system could help in getting over a lot of impediments like hydrophobicity(solubility is low) of major drugs lowers their therapeutic efficacy .The encapsulation of these drugs within the hydrophilic nanovehicles improves their solubility which in turn also enhances their bioavilability and their therapeutic efficiency.Further these drugs could also be encapsulated within the organic or lipid nanoparticles that prolongs their circulation time The half life of a therapeutic agent in the circulation is enhanced by manyfolds after encapsulating it within nano-liposomes. [**Hornan and Rieger 2001, Torchilin,2005**]. In the case of central nervous system cancers, Different drugs are unable to reach the tumor site by overcoming the blood– brain barrier.On the other hand,drug encapsulated nanoparticles have shown great efficiency in crossing this barrier and transporting the drug to the brain tumors.

The encapsulation of an anticancer drug within a nanoparticle and its subsequent targeting to a particular site helps in enhancing its therapeutic efficacy and also diminishes its toxicity to a

graet extent. [**Brannon-Peppas and Blanchette 2004; Ravikumar,2000**]. For example,the integrin conjugated lipid nanoparticle were able to deliver genes as well as drugs specifically to the angionenic blood vessel in case of tumor induced mice.This targeted nanoparticle was able to induce apoptosis and caused significant primaer and metastatic tumor regression [**Hood *et al* 2002**]. The primary attributes of the nanoparticle-mediated drug delivery is their potential to augment bioavailability ,enhance the sustained release of the drugs and also aid in specifically delivering the drug to the target site [**Dubin,2004;Dass and Su 2001; Drummond *et al* 1999**]. These delivery could be effectively integrated within the pulmonary therapies [**Courrier *et al* 2002**] in the form of emissaries of therapeutic genes[**Senior 1998**],and also in increasing the stability of the drugs that are prone to degeneration[**LaVan *et al* 2002,2003**].Different physiological charecteristics like the blood brain barrier,the divaricating pulmonary system and the compact epithelial junctions of the skin pose a steep impediment for the drugs to reach their desired location. These nano drug carriers parvade these obstacles and transport the drug to the target site. The maximum efficacy for pulmonary drug delivery is achieved by particles with size <100 nm [**Courrier *et al* 2002**]. The particles around 100 nm exhibit enhanced gastrointestinal absorption[**Desai,1996; Hussain *et al* 2001**] and the particles of 50 nm size display very high amount of trancutaneous permeation[**Kohli and Alpar, 2004**]. These nanoparticles traversing through the pulmonary tract could create a problem if they are exhaled. The use of larger, compartmental or multilayered drug carrier sytem could improve this pulmonary delivery. For example, the outer layers of the nanovehicles could be made in such a way that they erode while traversing the pulmonary tract. As this carrier penetrates furher, the biodegradable components of the carrier will fully degenerate triggering the release of the drugs into the lung. Biodegradable nanoparticles composed of gelatin and humanserum albumin function as promising pulmonary drug

carrier[**Brzoska** *et al* **2004**].

The nanoparticles are able to impregnate the tumor tissue through their characteristic tumor microvasculate which characteristically contains pores from 100-1000 nms. Hence these Nanoscale drug delivery architectures are able to penetrate tumors due to the discontinuous, or"leaky," nature of the tumor microvasculature, which typically contains pores ranging from 100 to 1000 nm in diameter.These pores provide the ideal entry points for the nanoparticles. These nanoparticles can be fabricated appropriately for attained effective drug delivery.

The different nanostructures like solid spheres, hollow spheres, tubes,porous particles, bifurcating structures are utilized for the purpose of drug delivery. The different types of fabrication methods are employed for the development of various kinds of nanoparticles.This encompasses vapor deposition [**Mamalis** *et al* **2004**], molecular self-assembly [**Zhang,2002**], nanomanipulation [**Hansma** *et al* **2004**], bioaggregation [**Mirkin** *et al* **1996**],photochemical patterning[**Cui,2003**], molecular imprinting[**Cui,2003**], layer-by-layer electrostatic deposition [**Ai,2002;Ai,2003**].

Nanovehicles that are commonly used for the delivery of therapeutics

The nanocarriers that are widely used as vehicles for the delivery of therapeutics comprises are usually conjugated with a targeting moiety.Different varieties of nanocarriers are widely used like liposomes, micelles, carbon nanotubes, dendrimers, nanoshells etc. The therapeutic agent can be attached with the nanocarrier through the proceeses of entrapping,covalent binding,encapsulation or adsorption. [**Vasir** *et al* **2005; Horn and Reiger,2001**]. The metal organic frameworks has been predominantly used in several biomedical applications such as adsorption, biosensing and catalysis [**Hu** *et al* **2014,Kennedy** *et al* **2013,Huxford** *et al* **2010**]. Among the differet metal organic frameworks, the Isoreticular Metalloorganic Frameworks (IRMOFs) have been widely utilized for the delivery of chemotherapeutic

agents [**Kundu** *et al* **2011,Rocca** *et al* **2014**]. Liposomes are composed of lipid bilayers where the core can be either hydrophilic or hydrophobic depending on the number of lipid bilayers[**Cui,2003;Ai,2002**]. Liposomes having a single lipid bilayer contain an aqueous core for entrapping the hydrophilic drugs. Liposomes that possesses more than a single bilayer can encapsulate the hydrophobic(lipid soluble)drugs [**Rosiak** *et al* **2003,Pricl** *et al* **2003**]. These liposomes are coated with polyethylene glycol (PEG) for ensuring their stability in the physiological conditions. [**RaviKumar,2000; Brzoska** *et al* **2004**]. The liposomes are coated with hyaluronan (HA) to prolong their circulation time in in vivo and augments its targeting to the overexpressing the HA receptors [**Panyam J and Labhasetwar, 2003;Panyam et al 2003**]. Liposomes can be conjugated with either antibodies or ligands for the purpose of active targeting to the selected target sites[**Nugent** *et al* **1998, Katare** *et al* **2003**]. They are biodegradable,non antigenic and have quite a high transport rate [**Lee** *et al* **2002**]. These liposomes can be fabricated pH responsive drug delivery and also thermotherapy [**Rosiak** *et al* **2003,Pricl** *et al* **2003,Namazi and Adeli,2004**]. The dendrimers are three dimensional divaricating structures with a multifunctional core which are either synthetic or are composed of elements like amino acids, sugars, and nucleotides[**Cloninger,2002**]. The characteristic branching points of the dendrimer aids in conjugating different hydrophobic and hydrophilic molecules such as therapeutic genes, drugs, anticancer agents, and so forth [**Prinz** *et al* **2003,Tao** *et al* **2003, Chen** *et al* **2004**].. Dendrimers in combination with other polymers like used in drug delivery studies typically incorporate one or more of the following polymers like polyamidoamine (PAMAM)[**Desai,1996;Cui** *et al* **2003**],melamine,poly (L-glutamic acid) (PG), polyethyleneimine(PEI) , poly (propylene imine) , and poly(ethylene glycol) (PEG) [**Mamalis et al 2004**]. Have been widely employed in the delivery of anticancer drugs.

 The micelles are spherical structures where molecules with a central hydrophobic core and the periphery is hydrophilic remain in close contact with the liquid environment just adjacent to the core.

Micelles are effective carrier for the delivery of lipid soluble drugs which are encapsulated in their hydrophobic core[**Li** *et al* **2004**]. The nanoparticles can be conjugated with appropriate ligands or antibodies for targeting them to the desired site.

Targeting ligands or antibodies can be attached to the surface [**Li** *et al* **2004**].,Fullerenes(also called bucky balls) and nanotubes.These nanotubes are fabricated to make them hydrophilic and are conjugated to the targeting ligands like peptides, nucleic acidsand therapeutic agents [**Weis** *et al* **2002**]. Nanotubes can be single walled or multiwalled [**Leoni** *et al* **2004**] and widely used for the delivery chemotherapeutic agents. The different polymers that are used includes poly (alkyl cyanoacrylates),poly(methylidenemalonate) and polyesters such as poly(ε-caprolactone),poly(lactic acid),poly(glycolic acid) and their copolymers like PLGA are most extensively researched due to their biocompatibility and biodegradability [**Mirakabad** *et al* **2014]**

Natural polymers as emerging carriers of therapeutics

Natural polymers are generally obtained from plant and animal kingdom. Most of the natural polymers are high molecular weight; water soluble polymers made up of monosaccharide units and joined by glycosides linkage [**Farooq** *et al* **2004**]. Gummy exudates of natural polymers such as protein, enzyme, muscle, fiber, and polysaccharide have been used to formulate various pharmaceutical products [**Farooq** *et al* **2014,Krishna** *et al* **2011**]. The well-known natural polymers are gelatin, Gum Acacia, aloe mucilage, guar gum, karaya gum, bhara gum, sodium alginate, locust bean gum, Hibiscus mucilageand linseed mucilage. These natural polymers are applicable in different pharmaceutical dosage forms like matrix controlled systems, microspheres, nanoparticles, buccal films and viscous liquid formulations [**Krishna** *et al* **2011**]. The specific application of natural polysaccharides in pharmaceutical preparation is to help in the processing of drug delivery systems during its manufacturing, protection, enhancement of stability, bioavailability and patient acceptability [**Malviya** *et al* **2011,Sujitha** *et al* **2012**]. Gums have various pharmaceutical applications such as suspending

agent for insoluble solid component in mixture, emulsifying agent for resin oils and adhesive in troche
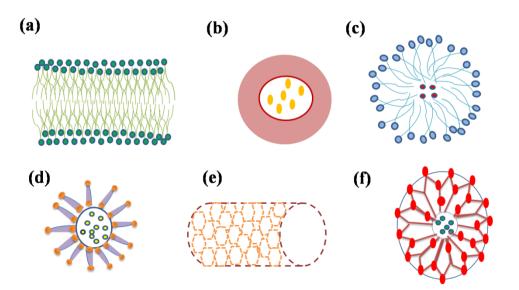
masses and pills [**Wang *et al* 2013**].

Fig-1.1.Structures of (a) liposome bilayer, (b) liposome, (c) micelle, (d) polymeric nanoparticles, and (e) dendrimer.

Targeted drug delivery Systems

The concept of designing a targeted delivery was first conceived by a microbiologist Paul Ehrlich. Targeted drug delivery involves the transport and accumulation of therapeutic agent to a desired site in without hampering the normal cells, thus enhancing the therapeutic efficacy of that agent . The primary features of targeted delivery are:

➢ It should be nontoxic, biocompatible, biodegradable, and exhibit physicochemical stability in both in vivo and in-vitro environments.

➢ The distribution of the drug should be limited to the desired site(cells,tissues,organs

or uniform capillary distribution.)

> The rate of drug release should be controllable and predictable.

> The drug distribution should be affected by the drug release.

> The amount of drug released by the system should be sufficient for therapy in the desired site.

> The drug leakage during transport should be restricted.

> The carriers must be biodegradable or readily eliminated from the body without exerting any hazardous effect and they should not temper the diseased site.

> The synthesis of these drug delivery system should be reasonably facile, high yielding and cost effective.

> Targeted drug delivery has been segregated into different categories considering the attributes of the target cells and also the type of targeting ligand.

Active Targeting.

In case of active targeting, nanoparticles containing the chemotherapeutic agents are designed in such a way such that they directly interact with the diseased cells. The active targeting is solely based on the recognition of the cellular biomolecules. Henceforth the nanoparticle are fabricated by attaching with the targeting agents in order to recognize the molecular patterns of the cancer cells which in turn helps in the delivery of the therapeutics to the desired site. These fabricated nanoparticles target the cancerous cells either through the process of ligand receptor interaction or by antibody-antigen recognition.These targeted delivery systems mainly comprise of the major components like, (i) a therapeutic agent (ii) a targeting ligand to enhance the penetration and (iii) a carrier. The cancerous cells have some unique attributes

like some specific receprors are overexpressed on their surface that help in discriminating them from the healthy cells .The conjugation of the specific ligands on the surface of nanoparticles enable them to specifically target the cancer cells.These targeted nanoparticles specifically bind with the receptors, undergo receptor-mediated endocytosis or phagocytosis, resulting in the internalization of the encapsulated drug and is rapidly cleared by the reticuloendothelial system (RES). Several endeavors have been made to exploit these ligand receptor interactions for the purpose of biomedical applications [**Dubin *et al* 2004**].

Passive Targeting- Nanoparticles can also target cancer through passive targeting.In cancerous cells there are anomalies in the blood vessels creating wide and leaky blood vessels near the endothelial which are promoted by angiogenesis [**Kannan *et al* 2004**]. The size of the pores in leaky endothelial cells ranges from 100 to 780 nm [**Pricl *et al* 2003;Namzi and Adeli 2004,Cloninger; 2002**].Hence the nanoparticles with that size range can easily penetrate through that endothelial pore[**Kannan *et al* 2004].**Henceforth the nanoparticles can be specifically to the capillary endolthelium for delivering drug to the desired organ. These nanoparticles can penetrate the tumor cells by passive diffusion or convection.The tumor cells are devoid of defined lymphatic system having leaky vessels which in turn facilitates the process of passive diffusion. Hence the drugs that penetrate with the tumor interstitum have prolonged retention times and this feature is regarded as enhanced permeability and retention (EPR) effect and this aids in the accumulation of drug with the tumor interstitium [**Panyam *et al* 2003,Nugent *et al* 1998**].

Inverse Targeting: This type of targeting attempts involves the circumventing of passive uptake of colloidal carrier by RES and therefore this targeting is regarded as inverse targeting. The inverse targeting is achieved by suppressing the normal functioning of RES through pre-injection of bare colloidal carriers or macromolecules like dextran sulphate

[Illium *et al*., 1989]. This leads to the saturation of RES which in turn hinders the normal defence mechanism.

Dual Targeting: In this targeting approach the vehicle possess intrinsic therapeutic activity which thus augments the therapeutic efficacy of a drug. For example, a vehicle having its own anticancer activity can be encapsulated with a anticancer drug and the cumulative synergistic effect of that drug was evaluated **[Gupta *et al*., 2000]**.

Double Targeting: When temporal and spatial methodologies are combined to target a carrier system, then that targeting strategy is regarded as double targeting. Spatial placement relates to targeting drugs to specific organs, tissues, cells.On the other hand, the temporal delivery refers to the regulation of the drug delivery rate to the desired site.

Folate receptor mediated drug delivery

Folic acid (FA) is a vitamin B complex which plays a pivotal role in cell viability through the biosynthesis of nucleic and amino acids. FA is a natural ligand of folate receptor (FR) and the binding affinity of FA for FR is approximately $K_d \sim 10^{-10}$ M (**Pan *et al* 2009**). Folic receptors are over-expressed in different types of primary as well as metastatic human cancers like the lung, the kidney, endometrium, ovary, colon, brain and triple negative breast cancer cells **[Zhang *et al* 2010,Lederman *et al* 2015, Cheung et al 2016]**.

Additionally the advancement in the stages/grades of cancer is closely associated with the increase in the density of FRs.Doxorubicin (DOX) is a widely used highly potent anticancer agent for a variety of tumors. But the doxorubicin also induces dose-dependent cardiotoxicity which restricts its successful clinical applications. Zhang et al successfully developed a targeted

drug delivery system of Doxorubicin by employing poly-(3-hydoxybutyrate-co-3-hydroxy octanoate) as a vehicle and Folic Acid as a active targeting ligand **[Zhang *et al*., 2010]**.

Types of vehicles	Anticancer drug	Targeting agent	Name of the polymers used	Outcome
Polymeric nanoparticles	Cystatin	Cytokeratin specific monoclonal antibody	Poly-(D-L-lactide co-glycolide) and polyethylene glycol	Prevent metastasis
Polymeric nanoparticles	Paclitaxel	Monoclonal antibody	Polylactic Acid	Selective targeting
Polymeric nanoparticles	Paclitaxel	Folic acid	Polylactic Acid and polyethylene glycol	Enhanced drug accumulation in tumor
Dendrimers	----	Folic acid	Polyamidoamine	Increased cellular uptake
Nanoshell	Docetaxel	Folic acid	Biodegradable polymer	Sustainable, controlled and targeted delivery
Dendrimer	Small interfering RNA(siRNA)	Luteinising hormone releasing hormone(LHRH)peptide	Poly(propyleneimine) and polyethylene glycol	High specificity
Nanoparticle	Paclitaxel	Folic acid	Poly-(D-L-lactide co-glycolide)	Inhibition of P-glycoprotein
Polymer micelle	Doxirubicin	Folic acid	PEG-co-(lactic co-glycolic acid)	Increased cellular uptake and cytotoxicity
Polymer micelle	Doxirubicin	Folic acid	PEG-poly(aspartate hydrazone)	Increased endocytotic cellular uptake

Table-1.1. Representing the different nanocarriers that has been formulated with positive results in the recent investigations.

Conclusions

These targeted nanoparticles face a steep challenge in releasing the drugs to the cancer cells since the hydrolytic enzymes residing in the lysosomes of the cells cause damage to both the nanoparticles and the encapsulated drugs.The tumors characteristically have a lower pH and this attribute can be exploited while designing the carriers.Thus the carriers which are responsive to the alterations in the pH and change their solubility at lower pH is quite conducive for drug delivery. A pH responsive nanoparticle typically comprises of a shell and a core The core-shell nanoparticles are designedth in a way that their lower critical solution temperature (LCST) is dependent on the ambient pH. The lower pH near the tumor cells causes a drastic alteration in the LCST of the coreshell nanoparticles which leads to its structural degradation that eventually stimultes the unleashing of the therapeutics.The conjugation of a targeting moity helps in specifically targeting these nanoparticles to the cancer cells. [**Neerman** *et al* **2004**]. A wide range of biodegradable nanoprticles have been designed from the the copolymers of poly(d,l-lactide-co-glycolide) which are endowed with the ability to deliver different therapeutic agents like DNA.The researchers have also developed thermoresponsive, pH-responsive by combining biodegradable poly (d,l-lactide) o, N-isopropyl acrylamide and methacrylic acid [**Mirakabad** *et al* **2014**]. These carrier systems are able to deliver the hydrophobic drug specifically to the cancer cells [**Tansey** *et al* **2004;Paleos** *et al* **2004**].

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