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Nano structure based drug delivery system: An approach to treat cancer

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Abstract

Nanotechnology has the potential to offer solutions to these current obstacles in cancer therapies, because of its unique size and large surface-to-volume ratios. Nanoparticles may have properties of self-assembly, stability, specificity, drug encapsulation and biocompatibility as a result of their material composition. Nanoscale devices have impacted cancer biology at three levels: early detection, tumour imaging using radio contrast nanoparticles or quantum dots; and drug delivery using nanovectors and hybrid nanoparticles. Other role of nanotechnology, in management of various diseases and also in drug resistance in leukemia by blocking drug efflux from cancer cells and induce efficient delivery of si RNA into lymphocytes to block apoptosis in sepsis and targeting tumors also. Nanocrystals labeling with immune cells can act as a platform technology for nanoimmunotherapy. This review addresses the advancement of nanoparticles in drug delivery and in cancer therapy.

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INTRODUCTION

Nanotechnology refers to the interactions of cellular and molecular components and engineered material, clusters of atoms, molecules, and molecular fragments at the most elemental level of biology.

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Such nanoscale objects with dimensions smaller than 100 nanometers can be useful by themselves or as part of larger devices containing multiple nanoscale objects.

Nanoscale devices have the potential to radically change cancer therapy for the better and to dramatically increase the number of highly effective therapeutic agents.

Although there have been significant advances in defining the fundamentals of cancer biology, this has not translated into similar clinical advances in cancer therapeutics. One area that holds great promise for making such advances is the area defined as cancer nanotechnology, which involves the intersection of a variety of disciplines, including engineering, materials science, chemistry, and physics with cancer biology. This multidisciplinary convergence has resulted in the creation of devices and/or materials that are themselves or have essential components in the 1–1000-nm range for at least one dimension and holds the possibility of rapidly advancing the state of cancer therapeutics and tumor imaging. The newly developing area of "nanohealth" is one hope in the treatment and cure of cancer that is still the second leading cause of death next to the cardiovascular diseases.[1]

The therapeutic agents used in cancer treatment are generally administered in the systemic circulation. The drug carrier, therefore, must overcome physiological barriers to reach the tumor cell in sufficient concentrations and to reside for the necessary duration to exert the pharmacological effect.

Delivery systems

The majority of nanotechnology-based devices useful for cancer therapeutics have been defined as nanovectors, which are injectable nanoscale delivery systems. Nanovectors offer the promise of providing breakthrough solutions to the problems of optimizing efficacy of therapeutic agents while simultaneously diminishing the deleterious side-effects that commonly accompany the use of both single chemotherapeutic agents as well as multimodality therapeutic regimens.[2]

Nanovectors are comprised of at least three constituents, which include a core material, a therapeutic imaging payload, and a biological surface modification, which aids in both appropriate biodistribution and selective localization of the nanovector and its cytotoxic and/or imaging agent. Although the first type of molecules used to enhance the selective localization and delivery of nanovectors were antibodies, more sophisticated recognition systems have been devised as a result of our expanding knowledge base in cancer biology.

The biological and molecular characteristics of human tumors of different origins continue to be defined and exploited for cancer therapeutics and tumor imaging, the significance of blood vessels that develop around actively growing tumors as potential therapeutic targets has only been widely recognized and found clinical utility within the past decade. The process of tumor-associated angiogenesis is now known to be an essential component of tumor expansion and metastasis. This realization and the accompanied potential for development of successful cancer therapeutics, as well as for tumor imaging strategies based on tumor-associated vasculature, has opened new doors for the application of nanotechnology in cancer therapeutics.[3]

Additionally, the molecules that drive the process of tumor angiogenesis may provide a means to gauge the timing and extent of individual patient responses to cancer treatment, which can also be monitored using nanovector approaches. One of the most important characteristics of nanovectors is their ability to be functionalized to overcome barriers that block access of agents used for treatment of tumors and for imaging of tumors and their associated

vasculature. These biological barriers are numerous and complex. One such barrier is the blood–brain barrier, which prevents access to brain malignancies, compounding the difficulties in their successful treatment.[4]

To achieve breakthrough advances in cancer therapeutics, there are two related and essential components which must be addressed. The first issue in successful use of nanovectors is recognition of the tumor and the second is the ability of the nanovector to reach the site of the tumor and associated blood vessels. The goal is to preferentially achieve high concentrations of a specific chemotherapeutic agent, a tumor imaging agent, and/or gene therapies at the site of tumors and associated vasculature. In addition, nanovectors must be able to deliver an active agent to achieve effective anti-tumor treatment, or tumor imaging, which is essential for tumor diagnosis and for monitoring the extent and timing of an individual patient's response to anti-tumor therapy.

The first nanotechnology-based approach to be used as a means of delivering cancer chemotherapy was liposomes, which is a type of nanovector made of lipids surrounding a water core. Liposomes are the simplest form of nanovector and their utility is based on the significant difference in endothelial structures, defined as fenestrations between normal vasculature and tumor-associated vessels. The increase in fenestrations in tumor neovasculature allows the preferential concentration of liposome-encapsulated anti-tumor agent in close proximity to the local tumor site, a phenomenon defined as enhanced penetration and retention (EPR), which is considered to be a characteristic of passive targeting of tumors.[5]

liposomes have been shown to be an effective means of delivering a diverse group of anti-tumor agents such as doxorubicin, as well as the poorly soluble drug paclitaxel. liposomes remain a versatile nanotechnology platform, historically serving as the prototypic nanovector. An approach that was first used with liposome nanovectors that has found utility with other types of nanovectors is PEGylation, a modification of liposome surface characteristics using poly(ethylene glycol) (PEG), resulting in what has been defined as "sterically stabilized liposomes." This PEG modification of liposomes provides protection against uptake by resident macrophages within the RES biobarrier, thus increasing the circulation time of liposome-encapsulated anti-tumor agent, resulting in significantly increased therapeutic efficacy. This approach to overcoming rapid uptake and destruction by resident macrophages within the RES by PEGylation has been used alone or in tandem with other modifications of liposomes to aid in avoiding or overcoming biobarriers and to more selectively localize nanovectors.[1,5]

liposomes have been shown to be an effective means for delivery of other agents such as genes and antisense oligonucleotides, and would allow access of such entities as small interfering RNA. As an example, peptides such as cell penetrating peptides (CPPs) have been used to modify liposomes, allowing them to be used as a means for intracellular drug delivery and delivery of proteins such as antibodies and genes, as well as providing a window for cellular imaging. In addition to liposomes as an example of the prototype of nanovectors, there are many others now available in the nanotechnology toolbox that are being investigated for use in cancer therapeutics and tumor imaging. Polymer-based nanovectors are a specific area of interest in cance therapeutics and a number of polymer-based nanovector systems are described. several categories, including polymer conjugates, polymeric nanoparticles, and polymeric micelles.[6]

Polymeric conjugates that have been investigated for use as nanovector systems for delivery of anti-tumor agents include the water soluble biocompatible polymer N-(2 hydroxypropyl) methacrylamide (HPMA). This polymeric conjugate has been targeted based on overexpression of hyaluronan (HA) receptors that are present on cancer cells. HPMA–HA polymeric conjugate drug delivery nanovector systems have been developed to carry a doxorubicin "payload" and have been shown to have selectivity for endocytosis of the targeted polymeric nanovector to breast, ovarian, and colon tumor cells, compared to an HPMA polymer with doxorubicin but lacking the HA targeting conjugate.18,19 Copolymer–peptide conjugates have also been developed as nanovectors using HPMA in combination with RGD targeting peptides, which molecularly target radiotherapeutic agents to tumor-associated vasculature, resulting in both antiangiogenic as well as anti-tumor activity.[2,3,7]

There are several approaches that exploit active targeting of long-circulating liposomes to tumor cells, where receptor-mediated internalization is strongly believed to bypass tumor cell multidrug-efflux pumps. These strategies utilize tumor-specific monoclonal antibodies or their internalizing epitopes, or ligands, such as folic acid, which are attached to the distal end of the poly(ethylene glycol) chains expressed on the surface of long-circulating liposomes. Nevertheless, with such approaches the delivery part is still passive and relies on liposome extravasation.[8,9]

Abraxanee is the only example of a regulatory approved (FDA, USA) nanoparticle formulation for intravenous drug delivery in cancer patients. It is paclitaxel bound to albumin nanoparticles, with a mean diameter of 130 nm, for use in individuals with metastatic breast cancer who have failed combination chemotherapy or relapse within 6 months of adjuvant chemotherapy. This formulation overcomes poor solubility of paclitaxel in the blood and allows patients to receive 50% more paclitaxel per dose over a 30-min period.[10]

Nanoparticles assembled from synthetic polymers have also received much attention in cancer drug delivery. One interesting example is doxorubicin-loaded poly(alkyl cyanoacrylate) (PACA) nanoparticles. In vitro studies have indicated that PACA nanoparticles can overcome drug resistance in tumor cells expressing multidrug-resistance-1-type efflux pumps. The mechanism of action is related to adherence of PACA nanoparticles to tumor cell plasma membrane, which initiates particle degradation and provides a concentration gradient for doxorubicin, and diffusion of doxorubicin across the plasma membrane following formation of an ion pair between the positively charged doxorubicin and the negatively charged cyanoacrylic acid (a nanoparticle degradation product). These observations clearly indicate that drug release and nanoparticle degradation must occur simultaneously, yielding an appropriate size complex with correct physicochemical properties for diffusion across the plasma membrane. Further developments with PACA nanoparticles include preparations that contain doxorubicin within the particle core and Nanotechnology for Cancer Therapy.[11]

General principles of Drug targeting to cancer Therapy

1. Passive Targeting

Passive targeting refers to the accumulation of drug or drug-carrier system at a particular site due to physicochemical or pharmacological factors. Permeability of the tumor vasculature increases to the point where particulate carriers such as nanoparticle can extravagate from blood circulation and localize in the tumor tissue. This occurs because as tumors grow and begin to outstrip the available supply of oxygen and nutrients, they release cytokines and other signaling molecules that recruit

new blood vessels to the tumor, a process known as angiogenesis. Angiogenic blood vessels, unlike the tight blood vessels in most normal tissues, have gaps as large as 600–800 nm between adjacent endothelial cells. Drug carriers in the nanometer size range can extravagate through these gaps into the tumor interstitial space. Because tumors have impaired lymphatic drainage, the carriers concentrate in the tumor, resulting in higher drug concentration in the tumor tissue (10-fold or higher) than that can be achieved with the same dose of free drug. This is commonly referred to as enhanced permeability and retention, or the EPR effect.[12]

2. Active Targeting

Active targeting to the tumor can be achieved by molecular recognition of cancer cells either via ligand–receptor or antibody–antigen interactions. Active targeting may also lead to receptor mediated cell internalization of drug carrier system. Nanoparticle and other polymer drug conjugates offer numerous opportunities for targeting tumors through surface modifications which allow specific biochemical interactions with the proteins/receptors expressed on target cells. For active and passive targeting of drug carrier systems, it is essential to avoid their uptake by the reticuloendothelial system (RES) so that they remain in the blood circulation and extravagate in the tumor vasculature. Particles with more hydrophobic surfaces are preferentially taken up by the liver, followed by the spleen and lungs. Size of nanoparticle as well as their surface characteristics is the key parameters that can alter the biodistribution of nanoparticles. Particles smaller than 100 nm and coated with hydrophilic polymers such as amphiphilic polymeric compounds which are made of polyethylene oxide such as poloxamers, poloxamines, or polyethylene glycol (PEG) are being investigated to avoid their uptake by the RES. To improve the efficacy of targeting cancer chemotherapeutics to the tumor, a combination of passive and active targeting strategy is being investigated where long-circulating drug carriers are conjugated to tumor cell specific
antibody or peptides. In addition to the above approach, direct intratumoral injection of the carrier system is feasible if the tumor is localized and can be accessed for administration of a carrier system.[13]

Applications of Nanoemulsions in Cancer Therapy

The advantages of formulating various lipophilic anti-cancer drugs in submicron O/W emulsion are obvious. The oil phase of the emulsion systems can act as a solubilizer for the lipophilic compound. Therefore, solubility of lipophilic drugs can be significantly enhanced in an emulsion system, leading to smaller administration volumes compared to an aqueous solution. In addition, because lipophilic drugs are incorporated within the innermost oil phase, they are sequestered from direct contact with body fluids and tissues. Lipid emulsions can minimize the pain associated with intravenously administered drugs by exposing the tissues to lower concentrations of the drug or by avoiding a tissue irritating vehicle.

Furthermore, incorporation of anti-cancer drugs in submicron emulsions (with droplet size of 50–200 nm) with long circulation properties are expected to enhance the tumor accumulation of the drug by passive targeting through the enhanced permeability and retention effect. It possible to enhance the tumor accumulation of nanoemulsions with appropriate modification of size or surface functionalization as previously discussed. Oil-in-water submicron emulsions appear to be a viable alternative for the intravenous administration of various lipophilic cytotoxic drugs.

Several groups of researchers have reported the submicron emulsion formulations of anti-cancer drugs for improved efficacy and/or reduced toxicity.

Paclitaxel, a highly potent anti-cancer agent initially extracted from the bark of the Pacific yew, was entrapped in lipid emulsion droplets with triolein as oil core and dipalmitoylphosphatidylcholine (DPPC) as the principal emulsifier. The emulsion was stabilized with polysorbate 80 and PEGdipalmitoyl PE. The incorporation of PEG-derivatized phospholipid is expected to enhance the in vivo circulation half life of the formulation, thereby enhancing the exposure of the drug to the targeted tumor mass.The formulation showed cytotoxicity against HeLa cells with an IC50 at 30 nM. An anti-tumor agent, valinomycin, was formulated in the emulsion form using the commercially-available Intralipid 10% soybean oil emulsion used in parenteral nutrition.

Evaluation of this formulation in vivo indicated that the emulsion formulation produced similarly shaped dose-response curve to that of an aqueous suspension, but the emulsion formulation required a 30-fold lower dose than the suspension to produce therapeutic similar effects.

In formulation of nanoemulsions, selection of the appropriate oil phase is important because most of the anti-cancer compounds exhibit poor solubility in the oil phase, especially those with highly lipophilic oils. Kan et al. determined the solubility of paclitaxel in various oils such as tributyrin, tricaproin, tricaprylin, corn, soyabean, cotton seed, and mineral oil, and they found that triacylglycerols with short fatty acid chains (tributyrin and tricaproin) were good solvents for paclitaxel with solubility of more than 9.00 mg/g as compared to other vegetable oils (range 0.14–0.23 mg/gm).

Another approach to enhance the oil solubility of the anti-cancer compounds is the chemical modification or prodrug formation. Prodrugs with increased oil solubility have been obtained with such anti-cancer drugs as teniposide, etoposide, camptothecin, and paclitaxel, whereas amphiphilic derivatives have been prepared from fluorodeoxyuridine. Esterification with long-chain fatty acids (i.e., oleic acid) has also been reported to increase the oil solubility of many anti-cancer drugs.[15]

Positively charged nanoemulsions in cancer therapy

Positively charged nanoemulsions systems are expected to interact with negatively charged cell surfaces more efficiently, and this aspect of the positively charged nanoemulsions has been explored for possibility of oligonucleotide delivery to cancer cells.50–53 Because oligonucleotides molecules display a polyanioinc character and present a large molecular structure, their ability to cross cell membranes remains very low.. The fate of both the pdT and the marker of the oil phase (cholesteryl oleate) were determined in the fluid (devoid of cancer cells) and in the P388/ ADR cell pellet. The results indicated that pdT in solution remained only in the fluid and did not associate at all with the tumor cells. However, pdT injected as an emulsion formulation was detectable in the cancer cells pellet even after 24 h and in very high proportions (up to 18% of the injected dose). When the area-under-the-curve values of the concentration versus time profiles for different formulations were compared, it was observed that RPR C18 formulations favored an increased association of pdT to the tumor cells compared to SA Because the distribution of the oil marker in the tumor fluid and the P388/ADR cell pellet could not be correlated with that of pdT, the authors postulated that pdT was probably not taken through the endocytosis of the oil droplets but by positive charges of the emulsion that probably increased membrane permeability and allowed the pdT molecules to more efficiently enter the cells..

Furthermore, the use of chitosan as a condensing agent (for DNA) and subsequent complexation with cationic emulsion composed of DC-chol enhanced the transfection efficiency in vitro compared to DNA/emulsion complexes with the same formulation with chitosan. In vivo study in mice showed that with chitosan enhanced emulsion complexes, the GFP mRNA expression was prolonged in liver and lung.[16]

Table1: Commercial Nanoemulsion Formulations

Drug	Brand name	Manufacturer	Therapeutic Indication
Propofol	Diprivan	Astra Zeneca	Anaesthetic
Dexamethasone Palmitate	Limethason	Mitsubishi Pharmaceutical	Steroid
Alprostadil	Liple	Mitsubishi Pharmaceutical	Vasodilator
Flurbiprofen axetil	Ropion	Kaken Pharmaceuticals	NSAID
Vitamin A,D,E,K	Vitalipid	Fresenius Kabi	Parenteral Nutrition

Neutron Capture Therapy of Cancer

Neutron Capture Therapy (NCT) is a binary radiation therapy modality that brings together two components that when kept separate, have only minor effects on the cells. The first component is a stable isotope of boron or gadolinium (Gd) that can be concentrated in tumor cells by a suitable delivery vehicle. The second is a beam of low-energy neutrons. Boron or Gd in or adjacent to the tumor cells disintegrates after capturing a neutron, and the high energy heavy charged particles produced through this interaction destroy only the cancer cells in close proximity to it, leaving adjacent normal cells largely unaffected.66 The success of NCT relies on the targeting of boron and Gd-based compounds to the tumor mass and to achieve desirable intracellular concentrations of these agents. At the present time, there are two targets with NCT, namely glioblastoma (malignant brain tumor) and malignant melanoma.

The formulation was designed based on the fact that LDL and high-density lipoproteins (HDL) are natural carriers of cholesteryl esters in the body, and certain human and animal tumor types have been shown to have elevated LDL-receptor activity primarily because the rapidly dividing cancer cells require higher amounts of cholesterol to build new cell membranes. It is expected that VLDL-resembling formulation may mimic the VLDL–LDL biological process for targeted drug delivery to cancer cells. Cell culture data showed sufficient uptake of BCH in rat 9 glioma cells (O50 mg boron/g cells).[17]

Dendrimer-Related Delivery Agents

Dendrimers are synthetic polymers with a well-defined globular structure. They are composed of a core molecule, repeat units that have three or more functionalities, and reactive surface groups.Two techniques have been used to synthesize these macromolecules:divergent growth outwards from the core,37 or convergent growth from the terminal groups inwards towards the core. Regular and repeated branching at each monomer group gives rise to a symmetric structure and pattern to the entire globular dendrimers. Dendrimers are an attractive platform for macromolecular imaging and gene delivery because of their low cytotoxicity and their multiple types of reactive terminal groups.

Boronated Dendrimers Linked to Monoclonal Antibodies

Boron Clusters Directly Linked to mAb

Monoclonal antibodies (mAb) have been attractive targeting agents for delivering radionuclides, drugs, toxins, and boron to tumors.Prior to the introduction of dendrimers as boron carriers, boron compounds were directly attached to mAbs.

Liposomes As Boron Delivery Agents

Liposomes are biodegradable, nontoxic vesicles that have been used to deliver both hydrophilic and hydrophobic agents . Both classical and PEGylated ("stealth") liposomes can increase the amounts of anti-cancer drugs that can be delivered to solid

tumors by passive targeting. Rapidly growing solid tumors have increased permeability to nanoparticles due to increased capillary pore size. These can range from 100 to 800 nm. In comparison, endothelial pore size of normal tissues, which are impermeable to liposomes, can range in size from 60 to 80 nm. In addition, tumors lack efficient lymphatic drainage, and consequently, clearance of extravasated liposomes is slow.

Modification of the liposomal surface by PEGylation or attachment of antibodies or receptor ligands, will improve their selective targeting and increase their circulation time. [6,7]

Boron Delivery By Dextrans

Dextrans are glucose polymers that consist mainly of a linear a-1,6-glucosidic linkage with some degree of branching via a 1,3-linkage. Dextrans have been used extensively as drug and protein carriers to increase drug circulation time. In addition, native or chemically-modified dextrans have been used for passive targeting to tumors, the RES or active receptor-specific cellular targeting.

To link boron compounds to dextrans, b-decachloro-o-carborane derivatives, in which one of the carbon atoms was substituted by –CH2CHOHCH2–O–CH2CHaCH2, were epoxidized and then subsequently bound to dextran with a resulting boron content of 4.3% (w/w). The modified dextran then could be attached to tumor-specific antibodies.–150 BSH was covalently coupled to dextran derivatives by two methods. In the first method, dextran was activated with 1-cyano-4-(dimethylamino)pyridine (CDAP) and subsequently coupled with 2-aminoethyl pyridyl disulfide. Then, thiolated dextran was linked to BSH in a disulfide exchange reaction. A total of 10– 20 boron cages were attached to each dextran chain.

In the second method, dextran was derivatized to a multiallyl derivative , which was reacted with BSH in a free-radical-initiated addition reaction. Using this method, 100–125 boron cages could be attached per dextran chain, suggesting that this derivative might be a promising template for the development of other HMW delivery agents.

Long-Circulating nanoparticles

Long-circulating nanoparticles can be created through surface modification of conventional nanoparticles with water-soluble polymers such as polyethylene glycol (PEG) or polyethylene oxide (PEO) .The hydrophilic nature of these surface modifiers minimizes the interactions between the nanoparticles and plasma proteins (opsonins), resulting in reduced uptake by the reticulo-endothelial system (RES). The major outcome of modification with PEG or other hydrophilic flexible polymers is a significant increase in circulation time, the advantages of which include maintenance of optimal therapeutic concentration of the drug in the blood after a single administration of the drug carrier, increased probability of extravasation and retention of the colloidal carrier in areas of discontinuous endothelium, and enhancement in targetability of the system by use of a target-specific ligand.

The protective action of PEG is mainly due to the formation of a dense, hydrophilic cloud of long polyethylene chains on the surface of the colloidal particle that reduces the hydrophobic interactions with the RES. The tethered or chemically anchored PEG chains undergo spatial conformations, thereby preventing the opsonization of particles by the RES of the liver and spleen and improving the circulation time of molecules and particles in the blood. The greater the flexibility of the polymer, the greater the total number of possible conformations and transitions from one conformation to another. Water molecules form a structured shell through hydrogen bonding to the ether oxygens of PEG. The tightly

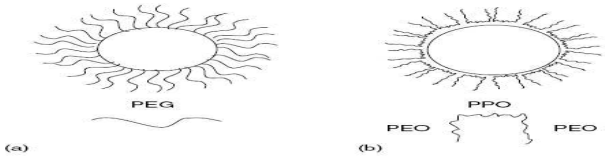
bound water around PEG chains forms a hydrated film around the particle and prevents protein interactions.

PEGylation may also increase the hydrodynamic size of the particles, decreasing their clearance through the kidneys, renal filtration being dependent on molecular mass and volume. This would ultimately result in an increase in the circulation half-life of the particles.

The size, molecular weight, and shape of the PEG fraction and the linkage used to connect it to the entity of interest determine the consequences of PEGylation in relation to protein adsorption and pharmacokinetics such as volume of distribution, circulation time, and renal clearance.

When formulated into colloidal particles, the PEG density on the colloidal surface can be changed by using PEG of appropriate molecular weight (PEG chain length) and molar ratio (the grafting efficiency). Longer PEG chains offer greater steric influence around the colloidal entity, similar to increased grafting density with shorter PEG chains. Longer PEG chains may also collapse onto the nanoparticle surface, providing a hydrophilic shield.

Besides PEG, other hydrophilic polymers, including polyvinyl alcohol, polyacryl amide, polyvinyl pyrrolidone, poly-[N-(2-hydroxypropyl methacrylamide], polysorbate, and block co-polymers such as poloxomer (Pluronicw) and poloxamine (Tetronicw), are also being used to modify the physicochemical properties of the colloidal carriers.[18]



Polyethylene Oxide-Modified Poly(b-Amino Ester) Nanoparticles

A representative biodegradable, hydrophobic poly(b-amino ester) (PBAE) with pH sensitive solubility properties was synthesized by conjugate addition of 4,4'-trimethylenedipiperidine with1,4-butanediol diacrylate, developed in Professor Robert Langer's lab at Massachusetts Institute of Technology. The paclitaxel-loaded nanoparticles (150–200 nm) prepared from PBAE were modified with Pluronicw F-108 (poloxamer 407), a triblock copolymer of polyethylene oxide/polypropylene oxide/polyethylene oxide (PEO/PPO/PEO).

The PPO segment of the triblock polymer attaches to the hydrophobic surface of the nanoparticles, and the hydrophilic PEO segment contributes to the stealth properties of the polymeric nanoparticles. The pH-sensitive nature of the particles prepared from PBAE has already been shown by in vitro release studies carried out in the presence of buffers of pH ranging from 5.0 to 7.4 and was found to rapidly degrade in a medium of pH less than 6.5. Therefore, these nanoparticles were expected to readily release their contents within the acidic tumor microenvironment and in the endosomes and lysosomes of the cells upon internalization. This was confirmed by the in vitro cellular uptake of the PEO–PBAE nanoparticles encapsulated with tritiated [3H]-paclitaxel by human breast adenocarcinoma cells .

The biodistribution of these PEO-modified PBAE nanoparticles was carried out by encapsulating a lipophilic form of the radionuclide indium-111 (^{111}indium oxine). Following tail vein injection in nude mice bearing a human ovarian xenograft, the radiolabeled PEO-modified PBAE nanoparticles were found to accumulate in the highly perfused organs such as the liver, spleen, and lungs with greater entrapment in the microvasculature of the lungs during the initial time points. The increasing concentrations in the kidney also indicate that the nanoparticles, once internalized, were disintegrated and eliminated through the kidney. The plasma half-life of the unmodified nanoparticles was reported to be one to ten minutes. By virtue of surface

modification with PEO, the PBAE nanoparticles were shown to have improved circulation times, resulting in a mean residence time in the systemic circulation of 21 h. The paclitaxel-encapsulated PEO–PBAEnanocarriers were found to deliver the drug efficiently to solid tumors, resulting in a 5.2-fold and 23-fold higher concentration of the drug at one hour and five hours post-administration relative to the solution form of the drug.From the tumor accumulation of the paclitaxel-loaded (3H-labeled) nanoparticles, it is evident that the pH-sensitive PEO–PBAE nanoparticle formulations can deliver significantly higher concentrations of the drug into the tumor than the solution form.[18,19]

Poly (ethylene oxide)-modified poly (3-Caprolactone) Nanoparticles

Poly (3-caprolactone) (PCL) is another biodegradable polymer that has been used to encapsulate hydrophobic drugs. Using this polymer, nanoparticles were prepared by solvent displacement in an acetone-water system in the presence of Pluronicw. The solvent displacement technique used for the preparation of PCL nanoparticles facilitates instant adsorption of PPO–PEO groups when theorganic solution of the polymer is introduced into aqueous solution containing the stabilizer. In addition, it also favors the encapsulation of hydrophobic drugs such as tamoxifen that could be dissolved along with the polymer in the organic phase, resulting in a high entrapment efficiency of greater than 90% at loading levels of 20% of the weight of the drug. The intracellular uptake of these nanoparticles in MCF-7 estrogen receptor-positive breast cancer cells and MDA-MB231 human breast adenocarcinoma cells was monitored at different time points using tritiated [3H]- tamoxifen. The results showed that the cell uptake followed saturable kinetics with most of the nanoparticles being internalized within the first 30 min of incubation.

The in vivo disposition of these PEO-modified PCL nanoparticles was completed in mice bearing MDA-MB231 xenograft breast cancer tumors as it is a well-characterized and simpler model compared to MCF-7 that requires estrogen priming for growth.

and drug-loaded, unmodified nanoparticles. At early time points (one hour), the nanoparticles modified with Pluronicw F-108 had greater concentration in the tumor with no significant difference in the concentration of the Pluronicw-modified formulations at six hours post-injection.

In a parallel study, the PEO-modified PCL nanoparticles were radiolabeled by a similar procedure specific to PEO–PBAE nanoparticles. The nanoparticles, encapsulated with [3H] tritium-labeled paclitaxel, were used to understand the change in concentration and localization of the drug in ovarian tumors (SKOV3). From the biodistribution studies, it was shown that the modification of PCL nanoparticles with PEO had extended the mean residence time to up to 25 h.

Hydrophobic drugs such as paclitaxel were found to have high plasma concentrations as a result of their protein binding capacity; however, they were cleared from the blood within 24 h. The circulation time of such drugs has been enhanced by encapsulating them in PEO–PCL nanoparticles that, in turn, has resulted in higher concentrations of the drug in the tumors. The PEO–PCL nanoparticles have resulted in an 8.7-fold increase in drug concentration at five-hour time points when compared to the solution form of the drug.

To potentially overcome MDR in ovarian cancer cell lines, C6-ceramide has been encapsulated along with paclitaxel into PEO-modified PCL nanoparticles. Upon treatment of the cells with paclitaxel, the MDR cell line SKOV3/TR exhibited 65.65G2. 16% viability at 1 mM dose; the the sensitive cell line SKOV3 showed 16.37G0. 41% viability at 100 nM dose. Co-treatment of these cells along with 20 mM C6-

ceramide in addition to paclitaxel (1 mM in the case of a resistant cell line and 100 nM in a sensitive cell line) resulted in a cell viability of 2.69G0.51% with the resistant cells and 7.38G1.25% with the sensitive cell lines, indicating a significant increase in cell death when compared to the paclitaxel treatment alone. Furthermore, the co-encapsulation of these drugs within PEO–PCL nanoparticles resulted in enhanced cell kill compared to the drugs alone.[10,18,20]

A 10 nM dose of paclitaxel, delivered in combination with ceramide in PEO–PCL nanoparticles, resulted in 63.98G4.9% viability, and the free drugs in solution at these doses did not provoke any cell kill in the resistant cell line. The use of these drug-loaded nanoparticles resulted in a 100-fold increase in chemosensitivity of the MDR cells. These results demonstrate the clinical use of PEO–PCL nanoparticles in overcoming MDR by combination therapy.[20]

PLGA as a Polymer for Nanoparticles

A number of different polymers, both synthetic and natural, have been utilized in formulating biodegradable nanoparticles. Synthetic polymers have the advantage of sustaining the release of the encapsulated therapeutic agent over a period of days to several weeks as compared to natural polymers which, in general, have a relatively short duration of drug release. The polymers used for the formulation of nanoparticles include synthetic polymers such as polylactide–polyglycolide copolymers, polyacrylates, and polycaprolactones, or natural polymers such as albumin, gelatin, alginate, collagen, and chitosan. Of these polymers, polylactides (PLA) and poly (D,L-lactide-coglycolide)(PLGA) have been most extensively investigated for drug delivery applications.

PLGA/PLA-based polymers have a number of advantages over other polymers used in drug and gene delivery, such as their biodegradability, biocompatibility, and approval by the FDA for human use. PLGA/PLA polymers degrade in the body through hydrolytic cleavage of the ester linkage to lactic and glycolic acid, although there are reports of involvement of enzymes in their biodegradation.

These monomers are easily metabolized in the body via Krebs' cycle and eliminated as carbon dioxide and water. Biodegradation products of PLGA and PLA polymers are formed at a very slow rate, and they therefore do not affect normal cell function. Furthermore, these polymers have been tested for toxicity and safety in extensive animal studies and are currently used in humans for resorbable sutures, bone implants and screws, contraceptive implants, and also as graft materials for artificial organs and supporting scaffolds in tissue engineering research. Long-term biocompatibility of these polymers was demonstrated by the absence of any untoward effects on intravascular administration of nanoparticles formulated using these polymers to the arterial tissue in pig and rat models of restenosis.[21]

Application Of PLGA/PLA Nanoparticles As Drug delivery Vehicles To Cancer Tissues

There are several studies regarding PLGA/PLA nanoparticles or some modification of these polymers for delivery of anti-cancer agents and other therapeutic agents.We have recently demonstrated increased efficacy of transferrin conjugated paclitaxel-loaded PLGA nanoparticles both in vitro and in an animal model of prostate carcinoma.Transferrin receptors are over-expressed in most cancer cells by two to tenfold more than in normal cells. We have demonstrated that transferring-conjugated nanoparticles have enhanced cellular uptake and retention than unconjugated nanoparticles. A single-dose intratumoral injection of transferrin conjugated nanoparticles in animal models of prostate carcinoma demonstrated

complete tumor regression and higher survival rate than animals that received either drug in solution or unconjugated nanoparticles.

The IC50 for paclitaxel with transferrin conjugated nanoparticles was fivefold lower than that with unconjugated nanoparticles or with drug in solution in PC3 24 and in MCF-7 cells. Kim et al. have demonstrated enhanced intracellular delivery of PLGA nanoparticles, which were surface-coated with cationic di-block copolymer, poly(L-lysine)–poly(ethylene glycol)–folate (PLL–PEG–FOL), in KB cells that overexpress folate receptors.In another study, paclitaxel-loaded PLGA nanoparticles, which were conjugated to wheat germ agglutinin (WGA), demonstrated greater anti-proliferation activity in A549 and H1299 cells as compared to the conventional paclitaxel formulations.

This enhanced activity of WGA-conjugated nanoparticles was attributed to greater intracellular accumulation of drug via WGA-receptor-mediated endocytosis of conjugated nanoparticles.33 Cegnar et al. have developed cystatin-loaded PLGA nanoparticles with the strategy of inhibiting the tumor-associated activity of intracellular cysteine proteases cathepsins B and L. In an in vitro study, cystatin-loaded PLGA nanoparticles demonstrated 160-fold greater cytotoxic effect in MCF-10A neoT cells than free cystatin. Similarly, interferon-alpha (IFN-alpha) loaded PLGA nanoparticles are being developed to improve the therapeutic efficacy of IFN-alpha while reducing its dose-related side effects.[18,21,22]

Polymer-Based Nuclear Imaging and Radiotherapy

With the appropriate delivery system, radioisotopes have a significant advantage over other therapy agents, namely, the emission of energy that can kill at a distance from the point of radioisotope localization. This diameter of effectiveness helps to overcome the problem of tumor heterogeneity because, unlike other molecular therapy (cell toxins, chemotherapy, etc.), not all tumor cells need to take up the radioisotope to eradicate a tumor.

There are also physical characteristics (type of particle emission, emission energy, half-life) of different radioisotopes that may be selected to enhance therapeutic effectiveness. For example, different isotopes deliver beta particulate ionization over millimeters (^{131}I) to centimeters (^{90}Y). Long-lived isotopes such as ^{131}I that remain within the tumor target may provide extended radiation exposure and high radiation dose, especially if there is progressive renal clearance and high target to non-target ratios. RGD peptides labeled with therapeutically relevant isotopes such as b-particle emitters have been investigated as potential angiogenesis targeted radiotherapy .

The chelation conditions for ^{90}Y and lutetium-177 (^{177}Lu) labeled RGD have revealed that time, temperature, pH, presence of trace metal contaminants, and stoichiometric ratio of chelator to isotope all have significant effects on the rate of chelation and radiolabeling efficiency. A major challenge in development of therapeutic radiopharmaceuticals is radiolytic degradation of radiolabeled products because of production of free radicals in the presence of a large amount of high energy ß-particles.

These conjugates may be particularly advantageous for cancer radiotherapy because the combination of polyvalent interaction and EPR effect would help to retain the conjugate in the tumor, enhancing the radiation dose.

The use of a water-soluble polymer (HPMA)-based conjugate of RGD peptide and the acyclic chelator

cyclohexyl-diethylenetriamine penta acetic acid for angiogenesis directed (^{90}Y) radiotherapy has been studied. After intravenous injection in prostate carcinoma . xenograft bearing SCID mice, the tumor accumulation of the conjugate peaked at 72 h post-injection, whereas the accumulation in other major organs significantly decreased during that period. A single injection of the ^{90}Y labeled conjugate at dose levels of 100 and 250 Ci caused significant reduction of tumor volume as compared to the untreated control that was evident from day 7 post-injection. [18,23]

Solid Lipid Nanoparticles fo Anti-Tumor Drug Delivery

Solid lipid nanoparticles (SLN) are colloidal particles of a lipid matrix that is solid at body.

temperature. Since their first introduction by Muller et al. SLN have attracted increasing interest as a carrier system for therapeutic and cosmetic applications. As a drug delivery system, SLN have been investigated in the last ten years for pharmacological and dermatological formulation development. They can be administered through a number of routes including parenteral, peroral, dermal and rectal.Improved bioavailability and targeting capacity have been observed and enhanced cytotoxicity against multidrug resistant cancer cells have been evidenced when SLN are used as the delivery vehicles.

SLN have been proposed as an alternative to other controlled drug delivery systems (CDDS) such as lipid emulsion, liposome, and polymeric nanoparticles as a result of their several advantages. For instance, in comparison to lipid emulsion, the solid lipid matrix of SLN makes sustained drug release possible. The solid lipids also immobilize drug molecules, thereby protecting the labile and sensitive drugs from coalescence and degradation, and reduce drug leakage that are commonly seen in many other CDDS such as liposomes. Compared with some polymeric nanoparticles, SLN are generally less toxic because physiological and biocompatible lipids are utilized. Meanwhile, all of the less toxic surfactants that have been applied to other CDDS are equally applicable for SLN preparation. Other appealing features of SLN include the feasibility for mass production, flexibility in sterilization, and avoidance of organic solvents in a typical SLN preparation process. It should be noted that SLN are also a versatile formulation. Both lipophilic and hydrophilic compounds can be encapsulated and delivered by SLN with modification in the formulation.[24]

The aforementioned useful qualities of SLN make them particularly attractive for the delivery

of cancer chemotherapeutic agents. Anti-tumor drugs, especially the cytotoxic compounds that are used in conventional chemotherapy, are unique when compared to other classes of drugs in a number of areas such as the strong toxicity that is typical of cytotoxic drugs often compromises their therapeutic effects; poor specificity of their drug action in general; and the frequent occurrence of drug resistance during chemotherapeutic treatment. These issues may all at least be partly tackled by delivering anti-tumor compounds with a suitable drug carrier system. SLN is potentially a valuable choice for this purpose. SLN may also be used for the delivery of these compounds to further improve the effectiveness of chemotherapy of cancer normally resistant to cytotoxic drugs.[25]

Conclusion

The amount of research in targeted, polymeric nanoparticles for cancer imaging and therapy has increased dramatically in the past 5–10 years. Seeing actual products using targeted therapies has no doubt fueled that work. In the next decade, we will certainly see products, whether with polymeric nanoparticles or some other type of delivery system,

using folate receptors and carrying imaging agents. All of these technologies, driven by the fields of fundamental immunology, biochemistry, polymer chemistry, and biomedical engineering, are bringing us closer to the time when cancer may be treated on an individual basis. One patient's diagnosis and treatment will be unique to her condition and will be the most effective treatment possible for her.

There are multiple factors affecting the delivery of drugs and genes to tumors. Factors such as blood flow, angiogenesis, microvessel density, interstitial pressure, macrophage activity, extracellular and intracellular components, and, most importantly, the physicochemical properties of the drug carrier play an important role in the transport of drugs and macromolecules to tumors.

Polymeric carriers could be modified using hydrophilic polymers such as PEG and PEO. This therapeutic strategy could be used to alter the passive/active targeting ability of the drug and gene carriers. However, the delivery of these newer agents is still a challenge, highlighting the necessity of additional research in this area.

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