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Role of nanotechnology in targeted drug delivery and imaging: a concise review

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Abstract The use of nanotechnology in drug delivery and imaging in vivo is a rapidly expanding field. The emphases of this review are on biophysical attributes of the drug delivery and imaging platforms as well as the biological aspects that enable targeting of these platforms to injured and diseased tissues and cells. The principles of passive and active targeting of nanosized carriers to inflamed and cancerous tissues with increased vascular leakiness, overexpression of specific epitopes, and cellular uptake of these nanoscale systems are discussed. Preparation methods—properties of nanoscale systems including liposomes, micelles, emulsions, nanoparticulates, and dendrimer nanocomposites, and clinical indications are outlined separately for drug delivery and imaging in vivo. Taken together, these relatively new and exciting data indicate that the future of nanomedicine is very promising, and that additional preclinical and clinical studies in relevant animal models and disease states, as well as long-term toxicity studies, should be conducted beyond the "proof-of-concept" stage. Large-scale manufacturing and costs of nanomedicines are also important issues to be addressed during development for clinical indications.

Key words: Nanotechnology; Targeted drug delivery; Imaging; Systemic administration

Nanotechnology is a rapidly expanding field today due to the multidisciplinary support from researchers in the academic, industry, and federal sectors. In 2001, the National Nanotechnology Initiative (NNI) [1], a multiagency US government program, was initiated. It supports research and development, infrastructure, education, and commercialization of nanotechnology. According to the latest update in March 2005, the 2006 NNI budget request for nanotechnology research and development across the federal government is $1.05 billion [1]. According to the NNI, nanotechnology is broadly defined as "the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel applications." The broad areas that are covered under nanotechnology include fundamental nanoscale phenomena and processes, nanomaterials, nanoscale devices and systems, instrumentation research, meteorology, standards for nanotechnology, nanomanufacturing, and societal studies of benefits and risks of nanotechnology.

In this review we focus on the role of nanotechnology in drug delivery and imaging. At present, 95% of all new potential therapeutics have poor pharmacokinetics and biopharmaceutical properties [2]. Therefore, there is a need to develop suitable drug delivery systems that distribute the therapeutically active drug molecule only to the site of action, without affecting healthy organs and tissues. Nanotechnology plays an important role in therapies of

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Table 1
Nanoscale systems for drug delivery

Drug delivery systems	Stage of development	Limitations of use	Examples of application	References
Liposomes	Marketed	Preparation steps have to be carefully controlled to achieve reproducible properties such as size and entrapment efficiency	Amphotericin B Daunorubicin Doxorubicin	[82] [83] [84]
Micelles				
Phospholipid	Preclinical	Limited stability in aqueous medium compared to other micelle types	Paclitaxel Camptothecin Diazepam	[16] [9] [101]
Pluronic®	Clinical Preclinical	Some monomers have not been tested in humans	Doxorubicin (SP1049C) Paclitaxel Tamoxifen Etoposide	[120] [15] [114] [115]
Poly (L-aminoacid)	Clinical In vitro	Immune response may increase with diversity in amino acids used. Biodegradability of poly(amino acids) requires validation	Doxorubicin (NK911) Antisense oligonucleotides	[128,129] [130]
Polyester	Preclinical	Polyester degrades by hydrolysis to produce acid metabolites that in excess may not be desirable	Paclitaxel Doxorubicin	[135] [136,137]
Nanoemulsions	Preclinical	High surfactant concentration of 20% and higher may be required in the formulation	Amphotericin B Paclitaxel Dexamethasone Benzathine penicillin G	[139] [140] [141] [142]
Nanoparticulate systems				
Drug nanocrystals	Preclinical	Polylmers and surfactants covering nanocrystal surfaces are required to provide stabilization against aggregation	Amphotericin B Etoposide, camptothecin, paclitaxel	[147] [150]
Polymer-based nanoparticles	Preclinical	Polyester degrades by hydrolysis to produce acid metabolites that in excess may not be desirable	Tamoxifen Cyclosporin-A Theophylline	[159] [160] [161]
Lipid-based nanoparticles	Preclinical	Enzymatic degradation in vivo can lead to production of undesirable metabolites such as stearic acid	Doxoribicin Camptothecin	[167] [168]
Ceramic-based nanoparticles	In vitro	Release of encapsulated drugs may be problematic	2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide	[171]
Albumin nanoparticles	Marketed In vitro	Validation of lack of immune reactivity maybe required	Paclitaxel DNA and antisense oligonucleotides	[14] [176,177]
Nanogels	Preclinical	Some polymers used may not yet be tested/used in humans	Oligonucleotides	[178]
Dendrimers	Preclinical In vitro	Positive charge on dendrimer surface may lead to toxicity and immunogenecity	Indometacin 5-fluorouracil Antisense oligonucleotides	[185] [192] [193]

the future as "nanomedicines" by enabling this situation to happen, thus lowering doses required for efficacy as well as increasing the therapeutic indices and safety profiles of new therapeutics. We define nanomedicines as delivery systems in the nanometer size range (preferably 1 to 100 nm) [1] containing encapsulated, dispersed, adsorbed, or conjugated drugs and imaging agents. This review will concentrate on delivery systems that can be systemically administered by parenteral routes. Nanomedicines have a size range that allows them to be injected without occluding needles and capillaries and are ideal for targeted drug delivery and medical imaging due to the pathophysiology of certain disorders such as cancer and inflammation. This review outlines selected nanoscale systems including liposomes, micelles [phospholipid-, Pluronic®- (BASF Corporation, Mount Olive, NJ), poly (amino acid)-, and polyester-based], nanoemulsions, nanoparticulate systems (drug nanoparticles, polymer-, lipid-, and ceramic-based, and albumin and nanogels), and dendrimers for drug delivery (Table 1) and imaging (Table 2). The reader is referred to another review published by Hughes [3] in an earlier issue of this journal on systems such as carbon-based nanotubes and metallic nanoshells. Therefore, these nanoscale systems will not be discussed in detail in this review.

The nanocarrier systems possess multiple desirable attributes. First, when drugs and imaging agents are associated with nanoscale carriers, their volumes of distri-

Table 2
Nanoscale systems for imaging

Drug delivery systems	Stage of development	Technique	Contrast agent/imaging agent/radiolabel	Limitations of use	References
Liposomes	Preclinical	SPECT MRI PET	^{99m}Tc Gadolinium ([2-18F]FDG)	Preparation steps have to be carefully controlled to achieve reproducible properties such as size and entrapment efficiency	[94] [196] [198]
Quantum dots/ nanocrystals	Preclinical	Optical/ fluorescence	Quantum dots Quantum dots-micelles Quantum dot-conjugates	Further safety studies are required because quantum dots are very stable	[201] [203] [202]
Magnetic nanoparticles	Clinical Preclinical Cellular	MRI	Iron oxide-dextran Iron oxide-polyacrylamide Iron oxide-SLN Iron oxide-insulin	Toxicity may occur due to cellular internalization and membrane disruption	[206] [208] [209] [207]
Dendrimers	Preclinical	MRI	Gadolinium	Positive charge on dendrimer surface may lead to toxicity and immunogenicity	[210,212]

MRI = magnetic resonance imaging; PET = positron emission tomography; SLN = solid lipid nanoparticles; SPECT = single photon emission computed tomography.

bution are reduced [4]. Nanoscale drug delivery systems also have the ability to improve the pharmacokinetics and increase biodistribution of therapeutic agents to target organs, which will result in improved efficacy [5-8]. Second, drug toxicity is reduced as a consequence of preferential accumulation at target sites and lower concentration in healthy tissues. Nanocarriers have been designed to target tumors and inflammation sites that have permeable vasculature. Targeting and reduced clearance increase therapeutic index and lower the dose required for efficacy. Third, many nanocarriers have the desirable advantage of improving solubility of hydrophobic compounds in the aqueous medium to render them suitable for parenteral administration. Fourth, delivery systems have been shown to increase the stability of a wide variety of therapeutic agents such as small hydrophobic molecules, peptides, and oligonucleotides [9-12]. Finally, nanocarriers composed of biocompatible materials [13-16] are investigated as safe alternatives to existing vehicles, such as Cremophor® EL (BASF, Mount Olive, NJ), that may cause hypersensitivity reactions and peripheral neuropathy [17,18].

Targeted drug delivery and imaging

Passive targeting

Passive targeting occurs due to extravasation of the nanoparticles at the diseased site where the microvasculature is leaky. Examples of such diseases where passive targeting of nanocarriers can be achieved are tumor and inflamed tissues. Tumor vascular leakiness is the result of increased angiogenesis and the presence of cytokines and other vasoactive factors that enhance permeability. Tumor angiogenesis is characterized by vessels with irregular diameters and branching, and tumors lacking defining structures of vasculature such as arterioles, capillaries, or venules [19]. Vascular endothelial growth factor (VEGF) and the angiopoietins are critical in regulating the balance between the leakiness associated with the defective endothelial linings of tumor vessels and vascular growth, maturation, and regression [20,21]. Elevated levels of bradykinin result in vasodilatation and enhance the extravasation of large molecules and their retention in tumors [22]. The increase in vascular permeability by VEGF and bradykinin is mediated by nitric oxide generation [23].

The majority of solid tumors exhibit a vascular pore cutoff size between 380 and 780 nm [24], although tumor vasculature organization may differ depending on the tumor type, its growth rate and microenvironment [24,25]. Therefore, particles need to be of a size much smaller than the cutoff pore diameter to reach to the target tumor sites. By contrast, normal vasculature is impermeable to drug-associated carriers larger than 2 to 4 nm compared to free, unassociated drug molecules [4,26,27]. This nanosize window offers the opportunity to increase drug accumulation and local concentration in target sites such as tumor or inflamed sites by extravasation, and significantly to reduce drug distribution and toxicity to normal tissues. Recently, researchers have also developed other approaches to increase local microvascular permeability and further enhance delivery to solid tumors and other targeted tissues. These include the use of physical energy such as hyperthermia [28] and ultrasound [29]. Recently, a genetic algorithm-based, area-coverage approach was developed for robot path planning to maximize drug delivery to a targeted area [30]. This approach allows devices such as microrobots to avoid unexpected obstacles (such as blood barriers, and important organs or tissues) and find a suboptimal path to achieve near-optimal energy consumption for drug delivery.

For passive targeting to be successful, the nanocarriers need to circulate in the blood for extended times so that there will be multiple possibilities for the nanocarriers to pass by the target site. Nanoparticulates usually have short circulation half-lives due to natural defense mechanisms of the body to eliminate them after opsonization by the mononuclear phagocytic system (MPS, also known as reticuloendothelial system). Therefore, the particle surfaces

Table 3
Selected examples of ligands used in active drug targeting

Targeting ligands	Targets	Examples of nanocarrier systems	References
Folate	Folate receptor	Liposomes Albumin nanoparticles	[51,97] [173]
Transferrin	Transferrin receptor	Liposomes Nanogels	[52-54] [178]
Insulin	Insulin receptor	Magnetic nanoparticles	[207]
Antibodies and their fragments			
Anti-HER2 and its fragments	HER-2 (or ERBB2) receptor	Liposomes	[95]
OX26 (anti-transferrin)	Transferrin receptor	Liposomes	[50]
323/A3	Epithelial glycoprotiens	Liposomes	[49]
Anti-Flk-1	Vascular endothelial growth factor receptor 2 (Flk-1)	Lipid nanoparticles	[48]
Anti-CD19	CD-19 epitope	Liposomes	[56]
Peptides			
Vasoactive intestinal peptides	Vasoactive intestinal peptide receptor	Phospholipid micelles, liposomes	[47,94,111]
RGD peptide	Cellular adhesion molecules such as integrins	Polymer nanoparticles	[45,46]
Luteinizing hormone-releasing hormone	Luteinizing hormone-releasing hormone receptor	Polymer nanoparticles	[44]
NGR peptide	Aminopeptidase N (CD13)	Liposomes	[43]
NGF peptide	Tyrosine kinase A (TrkA) receptors	Quantum dots	[205]
Gelatinase inhibitory peptide CTTHWGFTLC	MMP-2 and MMP-9 gelatinase	Liposomes and albumin nanoparticles	[42]
Aptamers	Proteins, peptides, enzymes, antibodies, various cell surface receptors, and small organic molecules	Polymer nanoparticles	[40,41]

need to be modified to be “invisible” to opsonization. A hydrophilic polymer such as polyethylene glycol (PEG) is commonly used for this purpose because it has desirable attributes such as low degree of immunogenicity and antigenicity, chemical inertness of the polymer backbone, and availability of the terminal primary hydroxyl groups for derivatization [31]. PEG-grafted liposomes, in the size range of 70 to 200 nm, containing 3 to 7 mol% methoxy-PEG-2000 grafted to distearoyl phosphatidylethanolamine (DSPE) or dipalmitoyl phosphatidylethanolamine, showed extended circulation half-lives of 15 to 24 hours in rodents and up to 45 hours in humans [32-34], whereas non-PEGylated liposomes had half-lives of 2 hours [35].

Drug molecules and imaging agents associated with nanocarriers can also target disease sites that have compromised barrier function and increased permeability due to the pathophysiology. The blood-brain barrier is an example where increased permeability has been reported in hypoxia-ischemia such as stroke; inflammatory and infectious diseases including multiple sclerosis, Alzheimer’s disease, septic encephalopathy, and HIV-induced dementia; and cancer [36-39]. Thus, nanotechnology can also be used to deliver therapeutic agents to the central nervous system with compromised blood-brain barrier for effective treatment. However, a precaution should be taken into account with nanomedicines for patients who have coexisting diseases with leaky vasculatures. This situation may result in passive distribution of therapeutics to multiple disease sites. However, this situation may in some cases be beneficial.

Active targeting

Localized diseases such as cancer or inflammation not only have leaky vasculature but also overexpress some epitopes or receptors that can be used as targets. Therefore, nanomedicines can also be actively targeted to these sites. Ligands that specifically bind to surface epitopes or receptors, preferentially overexpressed at target sites, have been coupled to the surface of long circulating nanocarriers [40-54]. Ligand-mediated active binding to sites and cellular uptake are particularly valuable to therapeutics that are not taken up easily by cells and require facilitation by fusion, endocytosis, or other processes to access their cellular active sites [55]. Active targeting can also enhance the distribution of nanomedicine within the tumor interstitium [4]. More recently, active targeting has been explored to deliver drugs into resistant cancer cells [56]. An important consideration when selecting the type of targeting ligand is its immunogenicity. For example, whole antibodies that expose their constant regions on the liposomal surface are more susceptible to Fc-receptor-mediated phagocytosis by the MPS [57,58]. Examples of targeting ligands and their targets are listed in Table 3.

Various methods have been employed to couple ligands to the surface of the nanocarriers with reactive groups. These can be divided into covalent and noncovalent couplings. Common covalent coupling methods involve formation of a disulfide bond, cross-linking between 2 primary amines, reaction between a carboxylic acid and primary amine, reaction between maleimide and thiol, reaction between hydrazide and aldehyde, and reaction between a primary amine and free aldehyde [59]. Noncovalent binding by physical association of targeting ligands to the nanocarrier surface has the advantage of eliminating the use of rigorous, destructive reaction agents. However, there are potential problems, such as low and weak binding and poor control of the reactions, and the ligands may not be

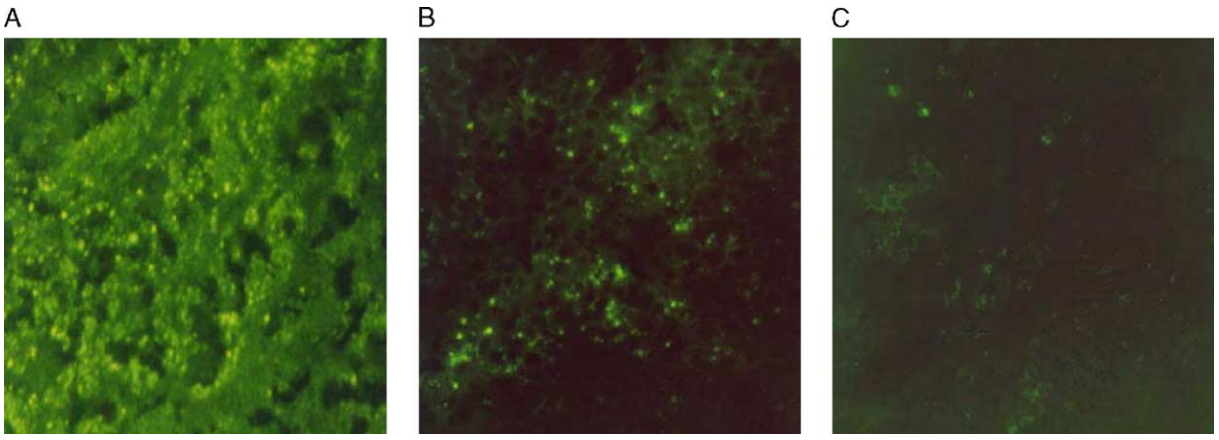

Fig 1. Comparison of the in vitro targeting ability of liposomes with conjugated VIP and noncovalently associated VIP to MNU-induced rat breast cancer tissues. **A,** BODIPY-Chol incorporating fluorescent VIP-SSL (with covalently attached VIP). **B,** BODIPY-Chol incorporating fluorescent SSL with noncovalently associated VIP. **C,** BODIPY-Chol incorporating fluorescent SSL without VIP. From Dagar et al [60].

in the desired orientation after binding. In our laboratory we used vasoactive intestinal peptide (VIP), a 28-amino acid mammalian neuropeptide, as a targeting moiety to cancer and inflamed tissues. We have compared the in vitro targeting ability of sterically stabilized liposomes (SSL) with vasoactive intestinal peptide (VIP) covalently bound (VIP-SSL) and noncovalently associated, as the targeting ligand to methyl nitrosourea (MNU)-induced rat breast cancer tissues [60]. Significantly more VIP-SSL were attached to the rat breast cancer tissue sections than SSL alone or SSL containing noncovalently associated VIP (Figure 1) [60]. Noncovalently associated VIP tended to dissociate from the SSL; therefore, covalently bound VIP was superior in terms of achieving greater targeting ability to VIP receptors on rat breast cancer tissues. However, noncovalent binding of VIP to the nanocarrier was preferred when delivering VIP as a therapeutic agent to VIP receptors of inflammatory cells in the joints of animals with rheumatoid arthritis [61].

Active targeting nanocarriers have a number of advantages over targeting ligand-drug conjugates. First, high concentrations of drug within the carrier can be delivered to the target cell when a ligand interacts with its receptor and large payloads of therapeutic agent relative to number of ligand binding sites can be achieved. This is especially advantageous in increasing tumor to background ratio in imaging. Second, the ligand is associated with the carrier, and the drug is not modified with the coupling of ligands. Drug activity may be compromised as the ligand-drug conjugate, or inactivated by the potentially aggressive coupling reaction. Third, numerous ligand molecules can be attached to the nanocarrier to increase probability of binding to target cells, particularly for those of lower binding affinities. Fourth, active targeting enables more efficient distribution of the carriers in the tumor interstitium and reduces return of drug back to the circulation due to high intratumoral pressure. Last, but also a very important point, is that when ligand is only attached to the carrier due to the small size of the conjugate, it can only extravasate at the disease site but not normal vasculature; therefore, the ligand cannot interact with the target epitopes of normal tissues and show side effects. In our laboratory we have shown that VIP receptors of normal cells are not accessible after intravenous injection when VIP was associated with a nanocarrier (Figure 2) [61]. Therefore, nanocarriers can play an important role in reducing toxicities of the drug and targeting ligand.

Cellular uptake of nanomedicines

Nanomedicines with a lower size range are preferable to those in the upper submicron and micron sizes to achieve longer-circulation half-lives (reduced MPS uptake) and more efficient cellular uptake (increased internalization). Intracellular uptake of particles can occur by various mechanisms, as described below.

Uptake by phagocytic cells

The MPS is made up of largely phagocytic cells such as macrophages. Generally, particles >1 μm generate a phagocytic response [62,63]. The uptake and transport of IgG-opsonized polystyrene beads of defined size ranging from 0.2 to 3 μm into murine macrophages were investigated by Koval et al [62]. They observed that phagocytosis uptake was size dependent; $<30\%$ of 0.2- to 0.75-μm particles compared to $>80\%$ of 2- and 3-μm particles were taken up. Also, to avoid substantial entrapment by hepatic and splenic endothelial fenestrations and subsequent clearance, carriers should not exceed 200 nm [31]. Besides size, which is the focus of this review, other properties of the nanocarriers, such as surface charge and chemistry, can also influence their uptake and subsequent clearance by the cells of the MPS.

Uptake by nonphagocytic cells

Internalization of particles by nonphagocytic cells such as tumor cells can also happen if particles are <500 nm [64]. Internalization of nanomedicines into the target cells can occur via a diverse range of endocytic pathways including phagocytosis, macropinocytosis, clathrin-mediated endocyto-

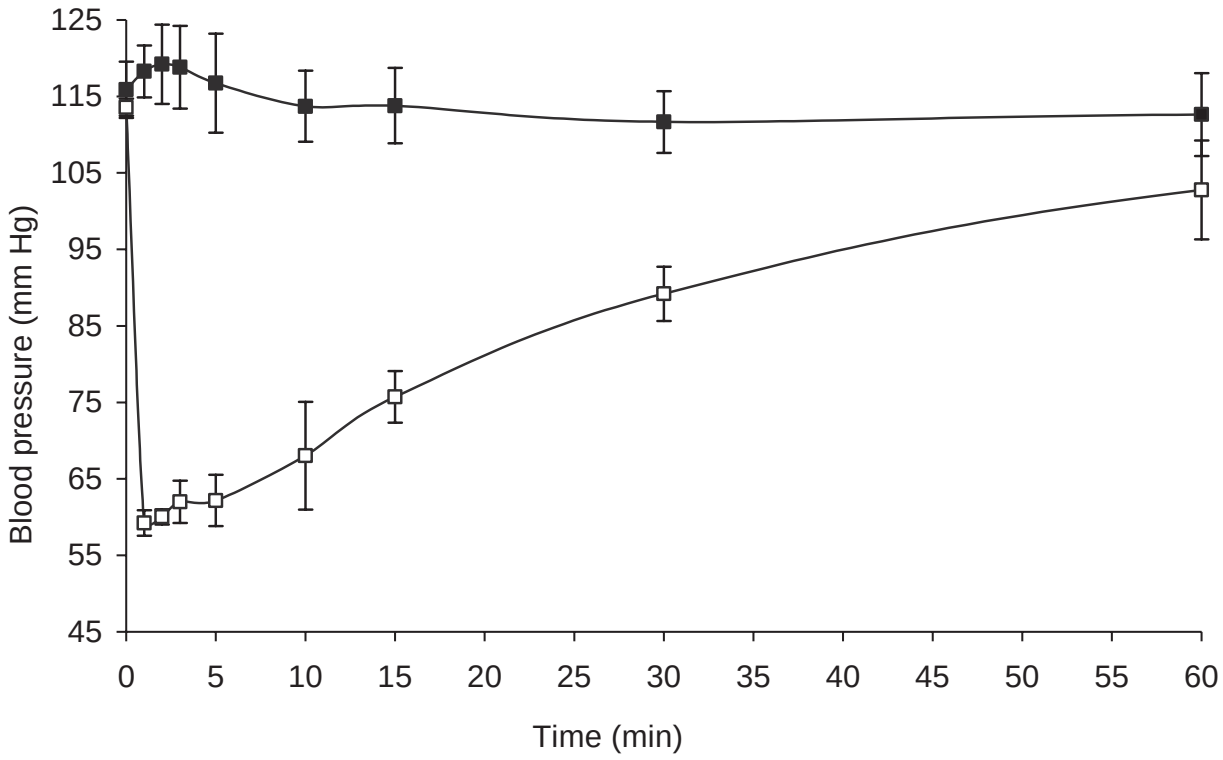

Fig 2. Effects of VIP micelles (■) and VIP alone (□) on mean systemic arterial blood pressure in mice. From Sethi et al [61].

sis, and non–clathrin-mediated (such as caveolae-mediated) endocytosis. Rejman et al [64] showed that as particle size increased, internalization was decreased. There was no cellular uptake of particles >500 nm.

Uptake by drug-resistant cancer cells

An area of research that is gaining interest is overcoming drug resistance in cancer chemotherapy by using nanoscale delivery systems. Major mechanisms that have been proposed include enhanced intracellular concentration of the drug by endocytosis [65], inhibition of multidrug resistance proteins by carrier component materials such as Pluronic block copolymers [66], adhesion of nanoparticles to the cell surface [65], promotion of other uptake mechanisms such as receptor-mediated cellular internalization [56,67], and increased drug concentrations at the vicinity of target cancer cells [65]. Furthermore, both drug and inhibitors of multidrug resistance proteins can be incorporated into the same carriers for simultaneous delivery to the cancer cells. For example, doxorubicin and cyclosporin A encapsulated in polyalkylcyanoacrylate nanoparticles have been demonstrated to reverse resistance synergistically [68].

Nanoscale systems for drug delivery

The nanoscale systems for drug delivery, the stages of their development, and examples of their application are summarized in Table 1.

Liposomes for drug delivery

Liposomes have been used as drug delivery systems since the 1960s [69]. Liposomes are defined as vesicles in which an aqueous volume is entirely surrounded by a phospholipid membrane [70]. Liposome size can vary from 30 nm to several micrometers, and can be uni- or multilamellar. Liposome properties have been extensively investigated and can vary substantially with desired size, lipid composition, surface charge, and method of preparation. Sethi et al describes various methods of liposome preparation and subsequent liposomal properties affecting drug activity [71]. Liposomes have to be smaller than the vascular pore cutoff (380 to 780 nm) to extravasate and reach solid tumors [24]. Vesicle size also plays a critical role in complement activation and MPS clearance of liposomes [4,72]. Vesicles larger than 100 nm require additional strategies to prevent surface opsonization.

A number of studies applied various surface modification approaches to classical liposomes to increase their circulation half-lives for effective passive targeting or sustained drug action. These approaches include incorporation of linear dextrans [73], sialic acid-containing gangliosides [74], and lipid derivatives of hydrophilic polymers such as PEG [75,76], poly-N-vinylpyrrolidones [77] and polyvinyl alcohol [78], to provide steric stabilization around the liposomes for protection from the MPS uptake.

Lipid composition can affect liposome interaction with therapeutic agents. We have successfully developed an SSL formulation of VIP that caused both enhanced and prolonged

activity of the peptide and decreased the mean systemic arterial pressure of spontaneously hypertensive hamsters [79,80]. This was due to the avid interaction of VIP with lipid bilayers composed of liquid-crystalline phospholipids and transitioning to its active α-helical form. On the other hand, there was limited interaction of VIP with gel phase 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine and egg phosphatidylglycerol (DPPC/ePG) liposomes due to greater energy requirement to separate the acyl chains for VIP to partition into the hydrophobic portion of the DPPC/ePG bilayer [81]. As a result, there was only modest potentiation of liposomal VIP-induced vasodilation in the intact peripheral microcirculation relative to similar concentrations of aqueous VIP.

In addition to the marketed liposomal products listed in Table 1 [82-84], liposomes are currently investigated for a variety of additional therapeutic agents. These include anticancer agents such as paclitaxel [13], camptothecin and its analogs [85-87], cisplatin [88]; antibiotics such as amikacin [89], vancomycin, and ciprofloxacin [90]; biologics such as antisense oligonucleotide [91], DNA, and small interfering RNA (siRNA) [92]; and muramyl tripeptide [93]. Liposomes have also been surface modified with active targeting ligands to improve delivery of therapeutics to target cells [94-97]. Recently, a multicomponent liposomal drug delivery system consisting of doxorubicin and antisense oligonucleotides targeted to MRP1 mRNA and BCL2 mRNA to suppress pump resistance and non–pump resistance, respectively, have been developed [96]. This liposomal system successfully delivered the antisense oligonucleotides and doxorubicin to cell nuclei, inhibited MRP1 and BCL2 protein synthesis, and substantially potentiated the anticancer action of doxorubicin by stimulating the caspase-dependent pathway of apoptosis in multidrug resistant human lung cancer cells.

Micelles for drug delivery

Micelles are self-assemblies of amphiphiles that form supramolecular core-shell structures in the aqueous environment. Hydrophobic interactions are the predominant driving force in the assembly of the amphiphiles in the aqueous medium when their concentrations exceed the critical micelle concentration (CMC) [98]. In this review we cover only the micelles that fall into the nanosize range that are formed with amphiphilic polymers. Typical classical surfactant micelles are not included in this review. Most nanosized micellar delivery systems are made up of amphiphilic polymers that consist of PEG and a low-molecular-weight hydrophobic core-forming block. Usually, the molecular weight of PEG (the outer corona component) is higher than the molecular weight of the hydrophobic core-forming block [99]. These types of micelles are generally smaller than 100 nm [9,99-101] and have CMC in the micromolar range [100,101]. Due to low monomer concentration in equilibrium with the micelles, these micellar delivery systems have reduced toxicity and are more thermodynamically stable to dilution compared to classical micelles formed with traditional surfactants that have CMCs orders of magnitude higher. Furthermore, nanosized micelles have polarity gradients from the highly hydrated corona to the hydrophobic core [100], and are used for solubilization of hydrophobic compounds of varying polarities by physical association with different regions within the micelles without drug modification. Finally, the biodistribution and pharmacokinetics of drugs such as doxorubicin, cisplatin, and paclitaxel are altered favorably, such as increased circulation half-life and tumor accumulation, when compared to free drug [102]. Micellar drug delivery systems can be divided into 4 classes that share a similar molecular architecture.

Phospholipid micelles

In contrast to typical phospholipids such as egg phosphatidylcholine, PEG-conjugated phospholipids such as DSPE-PEG are water soluble and self-assemble as nanosized micelles instead of bilayers. Recently, the focus of our laboratory is PEGylated phospholipid micelles that have the added advantage of simple and reproducible preparation compared to other lipid nanocarriers such as liposomes. PEGylated lipids with PEGs of molecular weights 2000 to 5000 were first discovered as hydrophilic anchors to liposomes that impart steric stabilization to avoid MPS uptake [32-34]. Thus, PEGylated phospholipid micelles can also avoid MPS uptake and have been demonstrated to have prolonged circulation times [12,103]; therefore, we describe them as sterically stabilized micelles (SSM). These types of micelles are biocompatible and relatively nontoxic [104,105]. PEG chains with molecular weight shorter than 1000 do not provide sufficient polarity to the phospholipid molecule to spontaneously form micelles. PEG chains longer than 5000 make the phospholipid head groups too bulky and the molecule too soluble. CMC of the PEGylated phospholipids ranged from 0.5 to 1.5 μM, with a higher CMC for longer PEG chain length [101]. To date we have demonstrated that a number of therapeutic agents can be solubilized and stabilized in SSM to be used as nanomedicines. These include paclitaxel [16], diazepam [101], camptothecin [9], and vasoactive intestinal peptide [106]. When drug solubilization in SSM was exceeded, a second population of drug self-aggregated particles (100 to 300 nm) besides drug SSM was observed [9,16,101]. We believe that these drug self-aggregated particles were sufficiently stabilized by PEGylated phospholipids on their surface against further precipitation and remain in the nanosize range, suspended in the clear aqueous dispersions. These PEGylated lipid-coated particles also have a promising future to deliver high drug concentration in a small volume, and to be further developed as nanomedicine as well. These systems were discussed in a recent review on nanosuspensions [107], and are further explored in the "Drug nanoparticles" section below.

We have further improved the solubilization potential of SSM by including a water insoluble phospholipid such as

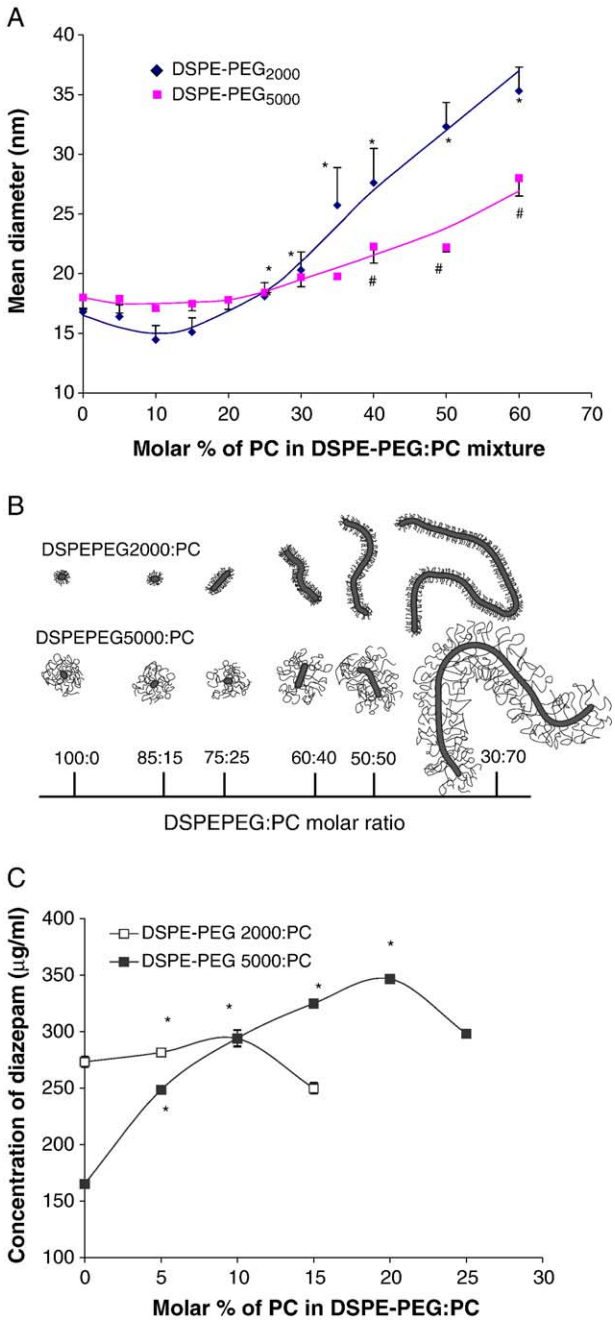

Fig 3. **A,** Size analysis of DSPE-PEG 2000:PC and DSPE-PEG 5000/PC. *, #, multiple-sized species. **B,** Schematic model of the relation between micelle composition and micelle structure for the DSPE-PEG2000/PC and DSPE-PEG5000/PC micelles. **C,** Effect of PC addition on solubilization potential of diazepam in sterically stabilized mixed micelles at room temperature; $n = 3$ experiments. *$P < .05$ compared to sterically stabilized micelles. From Ashok et al [101] and Arleth et al [108].

phosphatidylcholine (PC) to form sterically stabilized mixed micelles (SSMM) [16,101]. SSMM have similar nanosize as SSM but greater solubilization potential for paclitaxel due to the incorporation of PC [16]. Size and solubilization potential of SSMM varied with PEG chain length and PC content [101]. However, the mixed micellar system becomes heterogeneous in size when PC was added at and above 25%

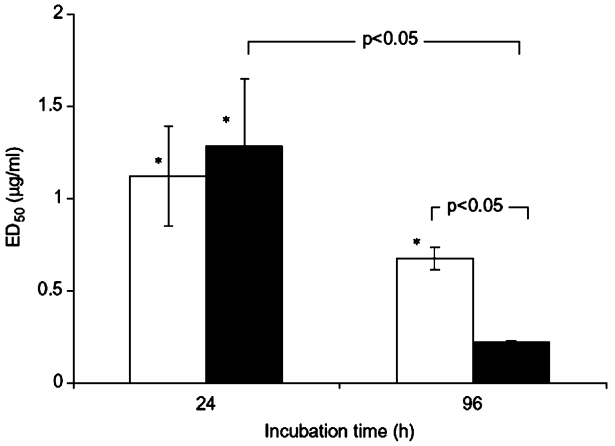

Fig 4. In vitro cytotoxicity of camptothecin (CPT) in 10% dimethylsulfoxide (DMSO) (*open*) and CPT-SSM (*closed*) to MCF-7 cells after 24 and 96 hours of incubation. ED50 of empty SSM controls were >2 mg/mL. Values are means and standard deviations; $n = 4$, experiments in triplicate. *No significant difference ($P > .05$) found between groups. From Koo et al [9].

for DSPE-PEG2000 and 40% for DSPE-PEG5000, respectively (Figure 3, *A* and *B*). Above these critical PC to PEGylated phospholipid ratios, a significant increase in aggregation number and formation of rod-like particles was observed [101,108]. Consequently, this phenomenon influenced the solubilization behavior of the SSMM (Figure 3, *C*). On the basis of small-angle x-ray scattering and small-angle neutron scattering analysis data, we have recently proposed a structural model for this SSMM system with a hydrophobic core, surrounded by a dense hydrophilic layer that is again surrounded by a corona of PEG chains in the form of Gaussian random coils attached to the outer surface [108]. The aggregation number of SSMM was around 90 for both DSPE-PEG2000/PC and DSPE-PEG5000/PC, below the critical PC to PEGylated phospholipid ratio [108].

We found that stability of therapeutic agents was enhanced when they are associated with SSM. We reported a 3-fold increase in camptothecin in vitro stability when it was associated with PEGylated phospholipid micelles [9], which subsequently increased camptothecin cytotoxicity (Figure 4). We also found that >83% of VIP associated with SSM remained stable after 7 days incubation in serum (37 °C), whereas 65% of free VIP degraded within 1 day [61]. PEGylated phospholipid micelles can be freeze-dried and reconstituted without the need of additional lyo- and cryo-protectants [9]. This is important in developing these phospholipid micellar systems further for clinical applications. SSMs can also be actively targeted by the attachment of ligands to the distal end of PEG [109-111]. In particular, we and others have found considerable success in surface modifying these micelles with VIP [111] and monoclonal antibodies (mAb 2C5] [109] for enhanced delivery of paclitaxel to breast cancer.

Pluronic micelles

Pluronics are block copolymers that consist of hydrophilic polyethylene oxide (PEO) (ie, PEG) and hydrophobic

polypropylene oxide (PPO) blocks arranged in a basic PEO_x-PPO_y-PEO_x structure (where "x" and "y" represent the number of times ethylene oxide [EO] and propylene oxide [PO] are repeated in the structure, respectively). Pluronics are available in varying molecular weights and ratios of EO to PO, thus providing an amphiphilic characteristic of a wide range of hydrophobicities, and Pluronic micellar formulations typically have combinations of 2 or more Pluronic types. The toxicity of Pluronic determined after intramuscular administration has been correlated to its hydrophobicity [112]. A number of comprehensive reviews and studies have been published on the application of Pluronic micelles (typically 20 to 100 nm) for drug delivery and imaging [99,100,113-115]. Extensive studies have also been conducted on the use of Pluronics to overcome multidrug resistance in chemotherapy in vitro [116-119]. Among the Pluronic micellar formulations, the formulation of doxorubicin and a mixture of L61 (PEO_{80}-PPO_{27}-PEO_{80}], and F127 Pluronic (PEO_{101}-PPO_{56}-PEO_{101}) (SP1049C) is most advanced and is now tested in Phase I clinical trial [120]. SP1049C had been reported to exhibit an acceptable safety profile with a maximum tolerated dose of 70 mg/m^2. The pharmacokinetic profile of SP1049C showed a slower clearance than conventional doxorubicin.

New polymers have been produced by chemical modifications. For example, Pluronics have been covalently conjugated with poly(acrylic acid) (PAA) that combines useful solubilization capability of the poloxamer surfactants and the pH-sensitivity and bioadhesive properties of the polyelectrolyte, PAA. These Pluronic-PAA micelles solubilized a higher concentration of camptothecin per PPO than nonconjugated Pluronic simple micelles, suggesting that camptothecin was not only solubilized by hydrophobic cores but also by the hydrophilic PEO-PAA shells of the micelles [121]. Recently, many studies have combined ultrasound with Pluronic micelles to achieve greater drug targeting to tumors [122,123]. Rapoport et al [122] demonstrated that in vivo delivery of doxorubicin in Pluronic micelles to rat tumors exposed to low-frequency ultrasound resulted in significant reduction in tumor size when compared with tumors not subjected to ultrasound. This was due to higher concentration of doxorubicin in the vicinity of the tumor due to ultrasonically activated drug release from the micelles. Cores of Pluronic micelles were chemically modified and cross-linked to prevent dissociation in blood when diluted below their CMC. For instance, Plurogels have a cross-linked, interpenetrating network of N,N-diethylacrylamide polymerized in the core of Pluronic P105 micelles [124].

Poly(L-amino acid) micelles

Poly(L-amino acid)-based micelles are investigated for their potential of pH-dependent release at tumor sites. Most solid tumors have pH values of less than 7.2 [125-127] due to higher rate of aerobic and anaerobic glycolysis in cancer cells compared to normal cells. Poly(L-histidine) (polyHis) micelles have been investigated as pH-sensitive anticancer drug carriers [126,128,129]. They were prepared by dissolving doxorubicin with blended block copolymers at different weight ratios of polyHis/PEG to poly(L-lactic acid) (PLLA)/PEG, and subsequent dialysis against alkaline buffer. The optimal formulation containing 25 wt% PLLA/PEG showed a desirable pH dependency, where 32 wt%, 70 wt%, and 82 wt% of doxorubicin was released at pH 7, pH 6.8, and pH 5, respectively. Besides imparting pH sensitivity to micelles, poly(His) also possessed fusogenic activity in endosomes to facilitate cytosolic delivery and enhanced doxorubicin micelles cytotoxicity to tumor cells [126] and resistance reversal in vivo [128]. Poly(L-amino acid) micelles have also been applied for delivery of antisense oligonucleotides [130]. The potential for immune response against poly(L-amino acid)s with 1 or 2 amino acids is low, but may increase with diversity [99]. Biodegradability of poly(L-amino acid)s requires validation, although it is commonly assumed. Micelle-forming block copolymers of poly(amino acid)-drug conjugates have also been formed and studied [131-133]; however, this involved chemical modification of the drug. Also decoupling of the drug from polymer at site of action could be a concern.

Polyester micelles

Polyester micelles are composed of polymers such as PEG-poly(lactic acid) (PLA), PEG-poly(lactic-co-glycolic acid) (PLGA), and PEG-poly(caprolactone) that are biocompatible, biodegradable, and Food and Drug Administration (FDA) approved for human use [99]. Lin et al [134] studied the effects of the type of lactone monomer, molar ratio of lactone/PEG, and molecular weight of the PEG chain on the performance and release behavior of drug-loaded micelles prepared by the dialysis method. The loading efficacy of indomethacin as a model drug in micelles increased as hydrophobic poly(lactone) chain length increased. On the other hand, drug release from more hydrophilic lactone such as PLA is faster due to weaker interactions between the drug and the poly(lactide) core. Shorter chain-length PEG4000 increased drug loading and slowed down drug release compared to PEG10000. Therefore, it was concluded that different hydrophobicity based on chemical structure of the poly(lactone) was responsible for different interaction strength between drug and micellar hydrophobic core, thereby affecting drug loading and release. Polyester micelles have also been investigated for delivery of paclitaxel [135] and doxorubicin [136,137].

Nanoemulsions for drug delivery

Nanoemulsions are dispersions of oil and water where the dispersed phase droplets are in the nanosize range and stabilized with a surface active film composed of surfactant and co-surfactant [138-142]. Nanoemulsions are transparent or translucent systems that have a dispersed-phase droplet size range of typically 20 to 200 nm [143], although in

earlier cases these systems have also been called microemulsions. Nanoemulsions are attractive as pharmaceutical formulations because they form spontaneously (ie, easy preparation), are thermodynamically stable, and optically transparent. The nanosize range of the droplets prevent creaming or sedimentation from occurring on storage and droplet coalescence. Structure of the nanoemulsion can affect the rate of drug release. Podlogar et al [138] used a variety of techniques, such as density, surface tension measurements, differential scanning calorimetry, and small-angle x-ray scattering to characterize the structure of a typical nanoemulsion system composed of varying water and isopropyl myristate with a constant amount of Tween 40 and Imwitor 308 at a mass ratio of 1. They found that a nanoemulsion containing >50 wt% water was oil-in-water (O/W) with strongly interacting oil droplets (5 to 14 nm). A nanoemulsion containing <20 wt% water was expected to be oil continuous with isolated water droplets (5 to 14 nm), a water-in-oil (W/O) nanoemulsion. In an O/W nanoemulsion, hydrophobic drugs are solubilized mainly in the oil droplets and will be released slowly (depending on the oil/water partitioning) due to hindered diffusion. Conversely, the diffusion of water-soluble drugs is less restrained and they will be released quickly. The reverse behavior is expected in W/O type. Finally, systems containing roughly between 20 and 50 wt% water will be both water as well as oil continuous, bicontinuous microemulsions. In such systems, relatively fast diffusion and release occur for both water-soluble and oil-soluble drugs. Nanoemulsions provide much longer oil-water contact area due to the nanosize droplet compared to classical emulsions, which facilitates drug release from the dispersed phase droplets.

In vivo pharmacokinetics of injectable nanoemulsions of vincristine have been investigated [144]. The formulation was composed of water, as the continuous and PEG-lipid with cholesterol as surfactants, and the oil phase was a vitamin E solution of oleic acid and vincristine. Vincristine was loaded into the oil phase of the nanoemulsion by increasing pH of the formulation to 7.4 to increase its lipid solubility. The emulsion mean particle size was 138 nm, and the preparation was stable because only 7.5% vincristine decomposition was observed after 1-year storage at 7°C in the dark. Plasma area under the curve of the vincristine nanoemulsion was significantly higher than free drug (soluble in aqueous NaCl) and in vivo biodistribution to tumor sites increased while distribution to MPS decreased. The researchers also found lower acute toxicity when animals were given vincristine microemulsion compared to free vincristine. This illustrated that nanoemulsions have the ability to decrease toxicity of anticancer agents by increased targeting to tumor sites. Another group of researchers incorporated poly(lactide-co-glycolide) to paclitaxel microemulsion for controlled release of paclitaxel [145]. Paclitaxel release rate was found to be retarded with increasing molecular weight of poly(lactide-co-glycolide). They concluded that in vivo antitumor action of this improved microemulsion system of paclitaxel (particle sizes of 45 to 270 nm) was greater than the original microemulsion formulation without poly(lactide-co-glycolide).

Other types of nanoemulsions are only kinetically stable; that is, they do not form spontaneously, which distinguishes them from thermodynamically stable microemulsions. Unlike spontaneous emulsions that require a high surfactant concentration (20% and higher), these nanoemulsions can be prepared by using lower surfactant concentrations. For example, a 20% O/W nanoemulsion may only require a surfactant concentration of 5% to 10% [143]. Methods to prepare nanoemulsions include lab-scale sonication, high-energy emulsification (using homogenizers), and low-energy emulsification whereby water is added to an oil solution of the surfactant. A low-dose amphotericin nanoemulsion system was intravenously administered to mice, rats, dogs, and monkeys at a dose of 1.0 mg/kg and compared to Fungizone® (Bristol-Myers Squibb, New York, NY) [146]. In contrast to Fungizone, amphotericin nanoemulsion showed a linear relationship between dose and area under the curve (AUC), indicating that efficacy can be more predictable from pharmacokinetics, and higher plasma concentrations of amphotericin were achieved with the nanoemulsion.

Nanoparticulate systems for drug delivery

Drug nanoparticles

Dispersion of drug particles in the nanosize range in an aqueous environment is an attractive approach for the delivery of water-insoluble drugs, and particularly for those not soluble in both water and nonpolar solvents and cannot be formulated by other approaches. Nanosuspensions of drug particles are commonly produced by 2 different methods. The first method of nanosuspension production involves the breaking down of bigger particles to nanosize using high-pressure homogenization of drug suspensions in the presence of surfactants such as Tween 80 and Pluronic F68 [147]. The second method involves crystallization building the nanoparticles up from the supersaturated solution state [107]. The solid state of nanosuspension is chemically more stable, gives high drug weight per volume loading, and is especially useful for therapeutic compounds that need high dosing. In the preparation, only maintenance of the drug in a crystalline state in the nanosize range is required but not in the dissolved state of the drug. Mean diameters of drug nanoparticles are typically 200 to 400 nm [148]. Rabinow [107] provides an excellent review on various nanosuspension systems for drug delivery.

Stabilizers such as polymers and surfactants covering the surface of the nanocrystals are required to provide steric or ionic stabilization of drug particles against aggregation. In our laboratory during the evaluation of PEGylated phospholipid micelles as solubilizers for water-insoluble drugs, we discovered the coexistence of PEGylated phospholipid-coated drug particles (100 to 300 nm), with drug-loaded

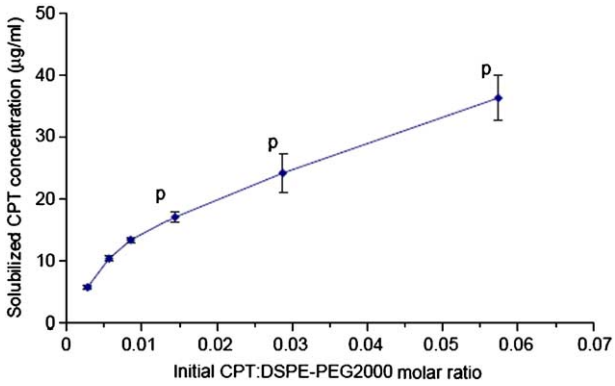

Fig 5. Solubilized camptothecin (CPT) concentrations as CPT-SSM with increasing initial CPT:DSPE-PEG2000 (fixed DSPE-PEG2000 concentration = 5 mmol/L). The p's indicate presence of an additional population of CPT self-aggregated particles besides CPT-SSM. Values are $n = 3$; error bars represent standard deviation. From Koo et al [9].

phospholipid micelles (Figure 5) [9]. These sterically stabilized particles did not precipitate during centrifugation at 13,000g for 5 minutes because of the steric stabilization imparted by the PEGylated phospholipids on their surface. Amphiphilic amino acid copolymers have also been investigated as a new class of stabilizers [149]. For successful polymer adsorption and size reduction to form nanocrystals, the mole fraction of the hydrophobic moieties of the stabilizers had to be at least 15 mol%. Comminution for only 5 minutes in the presence of these stabilizers was sufficient for nanocrystal preparation without any change in crystallinity, and drug nanocrystals (200 to 300 nm) were found to be stable up to 30 days without significant aggregation. Drug nanocrystals have been formed for amphotericin B [147], etoposide, camptothecin, and paclitaxel [150]. Nanoparticles of hydrophilic drugs such as dexamethasone phosphate have been successfully produced using supercritical carbon dioxide and encapsulated into poly(lactide-co-glycolide) macroparticles [151]. These drug nanoparticles (150 to 200 nm) when microencapsulated provided sustained drug release for 700 hours without the initial burst release observed when larger unprocessed drug particles (50 to 100 μm) were encapsulated. This was attributed to the more uniform dispersion of dexamethasone phosphate nanocrystals in the polymer matrix that allowed gradual release of the drug with polymer degradation. This supercritical carbon dioxide process is anhydrous and can be applied to produce nanocrystals of other hydrophilic drugs to be microencapsulated in sustained release formulations [151].

Solid nanoparticles

Nanoparticles can further be subclassified according to their composition: namely polymer-based, lipid-based, and ceramic-based materials, albumin nanoparticles and nanogels.

Polymer-based nanoparticles

These nanoparticles are made from copolymers to increase circulation half-life and reduce MPS uptake and inactivation. Nanoparticles of decreasing surface hydrophobicity have lower amounts of adsorbed plasma proteins and opsonins on their surfaces [152]. Poly(lactic acid) (PLA) poly(glycolic acid) (PGA), PLGA, poly-ε-caprolactone, and poly(methyl methacrylate) nanoparticles are the most widely studied [153-161] because they are biocompatible and FDA approved for human administration [162]. For instance, PLA and PLGA degrade by hydrolysis to lactic acid and glycolic acid metabolites that are eliminated via natural pathways in the body.

Methods involved in the preparation of polymer-based nanoparticles can be broadly divided into 2 general classes [163]. The first class involves polymerization of monomers, and the second class is based on the dispersion of preformed polymers [164]. Examples of the first are emulsion-polymerization [68] and dispersion-polymerization [165] methods. Examples of the second class are salting-out, emulsification-diffusion, and nanoprecipitation methods [164]. Besides being investigated as carriers for insoluble drugs, polymeric nanoparticles (eg, cross-linked polyvinylpyrrolidone nanoparticles) are also studied for delivery of hydrophilic drugs [166].

Lipid-based nanoparticles

Solid lipid nanoparticles (SLN) are a class of nanoscale carriers that have advantages such as the use of physiological lipids (ie, the solid lipid matrices can be composed of fats or waxes), avoidance of organic solvents in their preparation, protection of sensitive drugs from the external environment (eg, water), and controlled release of drugs [167,168]. SLN are produced by 2 different methods: "hot homogenization" of melted lipids at elevated temperatures or a "cold high-pressure homogenization" process [169]. The influence of lipid matrix, concentration, and size of SLN on murine peritoneal macrophages was investigated to determine possible cytotoxic effects and up-regulation of proinflammatory cytokines of these nanoparticles after intravenous injection [170]. Cytotoxicity was concentration-dependent and significantly influenced by the lipid matrix. Marked cellular cytotoxic effects were observed, with SLN consisting of stearic acid or dimethyl-dioctadecylammonium bromide at concentrations of 0.01%, whereas SLN consisting of triglycerides, cetylpalmitate, or paraffin did not exert major cytotoxic effects at the same concentrations. Cytotoxic effects were most likely attributed to products of enzymatic degradation, including free stearic acid.

SLN can be made sterically stabilized by incorporation of stearic acid-PEG 2000. The pharmacokinetic and tissue distribution effect of steric stabilization of SLN (SSLN) was investigated after intravenous administration of doxorubicin SLN, SSLN, and free drug into rabbits [167]. Area

under the curve increased with stearic acid-PEG 2000 amount, and all SLN formulations of doxorubicin resulted in lower distribution of drug to heart. For an excellent review on the use of lipid nanoparticles for the delivery of biotechnology drugs, see Muller and Keck [148].

Ceramic-based nanoparticles

Nanoparticles made of ceramic materials such as silica, alumina, and titania have several advantages over polymeric particles [171]. First, their preparations are simple, similar to the well-known sol-gel process, and require ambient temperature conditions. Second, the ceramic materials used are biocompatible, and their surfaces can be easily modified with different functional groups for ligand attachment. Third, the particles can be prepared to the desirable size, shape, porosity, and are extremely inert. The ceramic nanoparticles are sized at less than 50 nm. There are no swelling or porosity changes with pH, and they are not susceptible to microbial attack. Ceramic nanoparticles can protect absorbed or adsorbed molecules against denaturation induced by extreme pH and temperature.

Roy et al [171] developed ceramic-based nanoparticles as carriers of photosensitizing drugs for applications in photodynamic therapy. This was feasible because ceramic nanoparticles are highly stable and may not release encapsulated molecules even in extreme pH and temperature. However, although the photosensitizers may not be released, the porous matrices of the nanoparticles were permeable to molecular and singlet oxygen, thus maintaining the photodestructive action of the encapsulated drugs to cancer cells upon irradiation. The silica-based nanoparticles were prepared by controlled hydrolysis of triethoxyvinylsilane in micellar media, and found to be spherical and highly monodispersed (30 nm) [171]. The loss of fluorescence of the entrapped photosensitizing drug in aqueous media was prevented compared to free drug. These nanoparticles were actively taken up by tumor cells in vitro, and irradiation with visible light resulted in destruction of the cells.

Silica nanoparticles are recently used to form ternary complexes with DNA-dendrimer [172]. Silica nanoparticles are used because their dense nature can concentrate DNA at the surface of cells growing in culture. This allows efficient uptake of DNA by an endosomal-lysomal route. Transfection efficiency of the DNA-transfection reagent-silica nanoparticle complex was increased by a factor of 10 due to this mechanism.

Albumin nanoparticles

Albumin is a major protein component in serum. Albumin surface possesses several amino and carboxylic groups, which are available for covalent modification and drug or protein attachment. Albumin nanoparticles can be prepared by a desolvation/cross-linking technique, where dissolved albumin in water is desolvated by dropwise addition of ethanol and glutaraldehyde to induce albumin nanoparticle cross-linking over time [173]. A milestone in the clinical application of albumin nanoparticles was achieved in January 2005 when the FDA approved the use of paclitaxel albumin nanoparticles of ~130 nm (ABI-007 or Abraxane™, American Pharmaceutical Partners, Schaumburg, IL) for the treatment of metastatic breast cancer [14,174]. The overall response rate was 33% for Abraxane™, compared with 19% for Taxol® [175] ($P < .05$]. Median time to progression was 21.9 weeks for Abraxane™ versus 16.1 weeks for Taxol® ($P < .05$). Overall, side effects were fewer with Abraxane™, even though it delivered 50% higher dose of paclitaxel. The greater efficacy and lower toxicity of Abraxane™ could be attributed to passive targeting and greater retention of nanoparticles in cancer cells compared to free paclitaxel [175]. Also, the toxicity of the vehicle in Taxol® is eliminated with this formulation.

Albumin nanoparticles are investigated for DNA delivery because DNA-albumin can avoid opsonization and uptake by MPS encountered by positively charged complexes in vivo [176,177]. DNA-polyethylenimine (PEI)-albumin nanoparticles were less toxic than DNA-PEI complexes alone even at high concentrations [176]. This was attributed to lower surface charge of DNA-PEI-albumin nanoparticles resulting in less membrane irritation or damage. Wartlick et al [177] prepared albumin nanoparticles containing different antisense oligonucleotides, and optimized the process with respect to the amount of desolvating agent, stabilization conditions, and nanoparticle purification. They found that nanoparticles cross-linked with low amounts of glutaraldehyde degraded rapidly intracellularly. This resulted in a significant accumulation of the antisense oligonucleotides in cytosolic compartments of the tumor cells.

Nanogels

Existing solid nanoparticles may have disadvantages such as low drug-loading capacities and complicated preparation steps that involve organic solvents. Nanogels composed of flexible hydrophilic polymers in the nanosize scale can be made in the absence of drug. Upon equilibration or swelling in water, drug can be loaded spontaneously into the nanogel, resulting in reduction of the solvent volume, leading to gel collapse and formation of dense nanoparticles. Cationic nanogels have been applied to encapsulate negatively charged oligonucleotides spontaneously by ionic interactions [178,179]. The formation and stability of the DNA-nanogel complexes (<100 nm) were influenced by surface charge on the nanogel and the ionic strength of the solution [179]. High loading up to 50% of the macromolecules was possible. Vinogradov et al [178] observed at least two-thirds of the oligonucleotides remained associated with the nanogels after transport across bovine brain microvessel endothelial cells. Permeability of the oligonucleotides was enhanced up to 6-fold when compared to free oligonucleotides at fixed oligonucleotide concentrations of 5 μM. Further modification of the nanogel

structure with transferrin or insulin vector molecules resulted in 11- to 12-fold permeability increases. In vivo biodistribution indicated that 2.7% to 5.3% of the intravenously injected dose as oligonucleotide-loaded nanogels distributed to the intact brain compared to 0.2% achieved with the free oligonucleotides.

Dendrimer nanocomposites for drug delivery

Dendrimers are polymeric complexes that comprise a series of well-defined branches around an inner core with sizes (1 to 10 nm) and physicochemical properties similar to macromolecules [163,180]. Despite their large molecular mass (1000 to 800,000 kd) [181], dendrimers are structurally well defined and have low polydispersity, in contrast to many traditional polymers. Dendritic branching gives rise to semiglobular to globular structures and a high density of functionalities on the surface. Dendrimers can be functionalized with groups such as carbohydrates, peptides, and silicon to form glycodendrimers, peptide dendrimers, and silicon-based dendrimers, respectively [180,182]. The dendritic core is denoted generation "zero" (G0), and each "layer" between each focal point or cascade is called a "generation." Different linkages such as polyamines (eg, polypropylene imine, PPI dendrimer) or a mix of polyamides and amines (eg, polyamido amine, PAMAM dendrimer) can make up the dendrimer design. Dendrimer size can influence its extravasation across the endothelium into the surrounding interstitial tissue to reach the target sites. When size of the PAMAM dendrimers increased for G0 to G4 dendrimers from 1.5 to 4.5 nm, extravasation time across microvascular network endothelium increased exponentially [183].

Dendrimers can be synthesized by either divergent or convergent approaches [180]. In the former approach, the dendrimer is synthesized from the core as the starting point, and each successive generation will be built. However, this approach has the disadvantage of low yield because many reactions have to be conducted on a single molecule possessing a large number of equivalent reaction sites [184]. Furthermore, to avoid side reactions and drive reactions to completion, extremely large excesses of reagents are required in latter stages of synthesis, resulting in difficulties in purification. The convergent approach of synthesis [184] capitalizes on the symmetrical nature of the dendrimers, where the synthesis begins at the periphery of the final molecule and stops at the core where the dendrimer segments couple. Each synthesized generation of dendrimer can be subsequently purified.

Drug molecules can be associated with dendrimers in a variety of ways [182]. First, drugs can be physically encapsulated in the void spaces of the dendrimer interior by incubation. Second, dendrimer drug networks can be formed. Third, prodrugs can be formed from a linkage (either covalent or noncovalent) of the drug to the dendrimer surface. Passive targeting efficiency of PAMAM/indomethacin complex to inflammatory sites in arthritic rats was observed to be 2.29 times higher compared to free drug [185]. Quintana et al [181] further modified drug containing PAMAM dendrimers with folate to target tumor cells that overexpress the high affinity folate receptors. The folate-methotrexate-PAMAM dendrimer construct used at nontoxic polymer concentrations was 4-fold more cytotoxic to KB cells (cells derived from human epidermoid carcinoma) than free drug. Dendrimers have also been extensively investigated for gene delivery [186-189]. DNA can be complexed with intact PAMAM dendrimers that contain tertiary amines at branch points and primary amines at the termini. PAMAM dendrimer/DNA complexes (100 to 175 nm) have been characterized, and possible correlations between transfection efficiency with particle size/charge ratio and the relative affinity of the PAMAM dendrimer and DNA components have been suggested [187]. Dendrimer toxicity and immunogenic potentials should be considered when they are applied for drug delivery. Partial derivatization of the dendrimer surface, such as PAMAM dendrimer with PEG or fatty acids, helps to reduce toxicity and immunogenicity significantly due to a reduction/shielding of the positive charge on the dendrimer surface by the attached chains [190,191]. Dendrimers have been investigated for delivery of indomethacin [185], fluorouracil [192], and antisense oligonucleotides [193].

Nanoscale systems for imaging

The nanoscale systems containing contrast agents and radiopharmaceuticals for imaging, the stages of their development, and examples of their application are summarized in Table 2. In vivo imaging of these nanoscale systems can be carried out by using various types of imaging techniques, including single photon emission computed tomography (SPECT), positron emission tomography (PET), magnetic resonance imaging (MRI), fluorescence microscopy, computed tomography, and ultrasound. In this review we will focus on selected imaging modalities with documented applicability in vivo. Imaging plays an increasingly important role in disease detection and planning of therapy and surgery. Furthermore, clinical trials are depending more on imaging data to provide noninvasive, objective measures of therapy response.

Liposomes for imaging

Liposomes have been developed as carriers for a variety of contrast agents and radiopharmaceuticals (Table 2). We have focused on the development of actively targeted ^{99m}Tc-liposomes for SPECT imaging. Studies have shown that over expressed VIP receptors exist homogeneously in surgically resected human breast cancer and biopsies [194,195]. Our in vivo imaging studies showed that sterically stabilized liposomes (SSLs) encapsulating ^{99m}Tc-HMPAO with and without covalently attached VIP ligand accumulated significantly more in breast cancer than normal breast tissues (Figure 6) [94]. In particular, actively targeted VIP-SSL encapsulating ^{99m}Tc-Hexamethylpropyleneamine Oxime (^{99m}Tc-HMPAO)

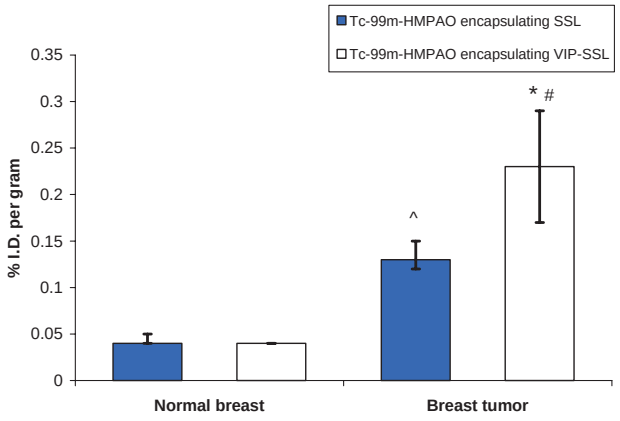

Fig 6. Accumulation of ^{99m}Tc-HMPAO encapsulating VIP-SSL (□) and ^{99m}Tc-HMPAO encapsulating SSL (■) in normal breast tissue and breast cancer tissue. *$P < .05$ compared to ^{99m}Tc-HMPAO encapsulating SSL in breast cancer; #$P < .05$ compared to ^{99m}Tc-HMPAO encapsulating SSL and VIP-SSL in normal breast tissue; ^$P < .05$ compared to ^{99m}Tc-HMPAO encapsulating SSL and VIP-SSL in normal breast tissue; $n = 5$, mean ± standard error of the mean. From Dagar et al [94].

accumulated significantly more in breast cancer than SSL without VIP. These data favorably support VIP as an ideal targeting agent to increase in vivo specificity and accumulation of liposomes in tumor sites over normal tissues. Liposomes being in the right nanosize range did not extravasate at the normal vasculature. Because normal vasculature is not leaky and VIP receptors exist only in the extravascular space, the imaging agent could not be distributed to the normal tissues to give high background signal [94].

Paramagnetic liposomes loaded with gadolinium (Gd) exhibited a 3-fold increase in relaxivity compared to conventional paramagnetic complexes gadoterate meglumine (Gd-DOTA) and gadolinium-diethylene triamine pentaacetic acid (Gd-DTPA) [196]. Therefore, these liposomes can be injected into mice at lower doses of 0.03 mmol/kg Gd corresponding to one-third the minimal clinical dose for MRI imaging. Furthermore, in comparison to the short half-life of most paramagnetic complexes, this study showed that agents associated with sterically stabilized liposomes exhibited good contrast enhancement in the tumor even 20 hours after injection. Our review on the use of liposomes in ultrasound and scintigraphic imaging provides more examples [197].

Liposomes composed of dipalmitoylphosphatidylcholine, cholesterol, and palmityl-D-glucuronide (PGlcUA) in a molar ratio of 4:4:1 were prepared in the presence of 2-[18F]fluoro- 2-deoxyglucose to observe real-time liposomal trafficking by PET [198]. Time-activity curves indicated that the liposomes (<200 nm) transiently accumulated in the liver right after the injection but they re-entered the bloodstream with time. The majority of the liposomes remained in the bloodstream until they finally accumulate in the tumor tissues. PGlcUA was effective in increasing the circulation half-life of the liposomes and, compared to control liposomes without PGlcUA, these liposomes had lower liver and higher tumor accumulation.

An interesting study showed that it was feasible to image optical and PET reporter gene expression in the same animal using the charge-coupled device (CCD) camera and micro-PET, respectively, after a single intravenous injection of 1,2-Dioleoyl-3-Trimethylammonium-Propane (DOTAP): cholesterol DNA liposome complexes [199]. High levels of 2 different reporter gene expressions predominantly in the lungs can be simultaneously observed by optical and PET imaging to be time- and dose-dependent after the tail vein injection. There was a good correlation between the in vivo bioluminescent signal and the ex vivo firefly luciferase enzyme activity in different organs. This noninvasive, clinically applicable imaging method will greatly aid in the optimization of future gene therapy approaches for cancer.

Quantum dots for imaging

Quantum dots (QD) are crystalline aggregates of a few hundred atoms of elements from group II-VI or III-V of the periodic table, coated with an insulating outer shell of a different material. They are particles with physical dimensions smaller than the excitation Bohr radius [200]. QD have a future in imaging because their size range (2 to 8 nm) confers unique optical and electronic properties such as tunable fluorescent emission by varying particle size or composition. In vivo imaging of QD after injection involves excitation using a spatially broad source (long wavelength) and capture of the resulting fluorescence with a sensitive CCD camera. Optical imaging equipment costs are lower than competing technologies such as MRI. By using QD-labeled tumor cells, it was possible to follow their paths after intraveneous administration as they extravasated into the lungs [201]. This can potentially help to study metastatic tumor cell extravasation. However, toxicity of stable QD is yet to be determined.

QD are mostly synthesized in nonpolar solvents. Therefore, their hydrophobic surface ligands must be replaced by amphiphilic ones to solubilize them in aqueous medium. Akerman et al [202] modified ZnS-capped CdSe QD to render them water soluble and coated them with targeting peptide sequences (GFE, F3, and LyP-1). QD coated with GFE, F3, and LyP-1 peptides that preferentially binded to lung, blood vessels, and lymphatics in tumors, respectively, were directed accordingly when administered intravenously to mice. Adding PEG to the quantum dot coating reduced MPS uptake. QD are also associated with nanoscale systems such as phospholipid micelles [203] and silicon nanospheres [204] to improve their solubilization and reduce accumulation in liver and bone. Currently, our laboratory is exploring the cellular internalization process of phospholipid micelles through VIP receptors by imaging micelles loaded with quantum dots in cell culture (unpublished data). Ligands and other effector compounds have been conjugated to the quantum dot surfaces to form imaging probes with specificity to induce cellular pharmacologic responses. Nerve growth factor (NGF) peptides conjugated to QD have been demonstrated to retain bioactivity and interact with their receptors, the TrkA

receptors to initiate neuronal outgrowth and differentiation in PC12 cells [205].

Magnetic nanoparticles for imaging

Colloidal iron oxide formulated with dextran is clinically used as MRI contrast agents [206]. However, current research is under way to further increase tissue targeting and reduce toxicity of these nanoparticles due to cellular internalization and subsequent cell membrane disruption. A study showed that covalent coupling of insulin on the iron oxide nanoparticle surface decreased internalization of the nanoparticles in vitro [207]. This was attributed to attachment of the insulin-coated nanoparticles to the cell surface receptors, thus preventing internalization and reduced cellular toxicity. Multiple crystals of iron oxide have been embedded in nanoparticle matrices such as polyacrylamide with surface PEG [208] and solid lipid nanoparticles [209]. Iron oxide in such polyacrylamide nanoparticles when injected into rats bearing orthotopic 9L gliomas had significant increases in relaxivity and circulation half-life up to 3-fold [208].

Dendrimer nanocomposites for imaging

Smaller dendrimer-based Gd MRI contrast agents (molecular weight <60 kd) have been developed to overcome the prolonged retention times and toxicity of larger dendrimer and albumin MRI products [210-212]. These smaller dendrimer-based MRI agents were more quickly excreted by the kidneys and were able to make visualization of vascular structures better than Gd-DTPA due to less extravasation [211]. Second and third generation of both diaminobutane core PPI and polyamidoamine (PAMAM) dendrimers were compared [210,212]. The relaxivity of the PPI agent was 50% greater than the corresponding generation PAMAM agent. Second-generation PPI increased the contrast enhancement 1 hour after injection into rats of T1-weighted images by 52% in comparison to a Gd-HP-DO3A (ProHance®, Bracco Diagnostic Inc., Princeton, NJ) reference standard [212].

Conclusions and future directions

The field of nanomedicine has a bright future with the emergence of several promising approaches for delivery of therapeutic agents and imaging using the advantages of the nanoscale carriers. Various initiatives from both the federal agencies as well as industry support the continual research into the application of nanotechnology to improve drug delivery and molecular imaging. However, it is also recognized that as research moves toward developing smaller and smaller devices and agents, larger multidisciplinary teams are needed for success. Collaboration also requires greater communication between various disciplines, including medicine, engineering, materials science, information technology, and physics, to expand on existing knowledge. Future studies should also aim to address a number of challenges faced in nanomedicine application. First, additional preclinical and clinical studies in relevant animal models and disease states should be performed to substantiate proof of concept. Second, a number of these novel nanoscale systems still lack safety data, in particular long-term toxicity studies should be carried out beyond "proof-of-concept" studies. Third, issues related to scale-up and manufacturing should be addressed. Long-term storage stability is another requirement when considering these systems for clinical application. Finally, the cost of these nanomedicines should be in an acceptable low range to be successful in the clinics.

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