Overview

Nanomedical engineering: shaping future nanomedicines

Dandan Luo,[1] Kevin A. Carter[1,2] and Jonathan F. Lovell[1,2]*

Preclinical research in the field of nanomedicine continues to produce a steady stream of new nanoparticles with unique capabilities and complex properties. With improvements come promising treatments for diseases, with the ultimate goal of clinical translation and better patient outcomes compared with current standards of care. Here, we outline engineering considerations for nanomedicines, with respect to design criteria, targeting, and stimuli-triggered drug release strategies. General properties, clinical relevance, and current research advances of various nanomedicines are discussed in light of how these will realize their potential and shape the future of the field. © 2014 Wiley Periodicals, Inc.

How to cite this article:
WIREs Nanomed Nanobiotechnol 2015, 7:169–188. doi: 10.1002/wnan.1315

INTRODUCTION

Nanomedical engineering involves the manipulation of matter in the size range of 1–1000 nm for medical applications. With the concurrent rise of the fields of biomedical engineering and nanotechnology, the intersected field of nanomedical engineering has grown remarkably in the past decades.[1] Nanomedicine refers more specifically to medicines with nanoscale size, properties or features that are administered for patient benefit. There are numerous types of nanomedicines, but they generally can be categorized as therapeutic agents, medical imaging agents, or carriers for drug delivery. Nanomedicines also hold potential for disease detection and diagnosis.[2] Nanomedical engineering seeks to rationally design and develop these, often with emphasis on size, shape, degradation, and surface properties. An understanding of the biological properties of both the target tissue and the physiological route that must be traveled by the nanoparticles to reach that tissue is beneficial.

A large research focus of nanomedicines has been placed on cancer treatments,[3] although there have also been intensive research efforts spent on other health problems including cardiovascular disease[4] and respiratory diseases[5] among many others.[6] Cancer chemotherapy drugs are somewhat unique in that they are often intended to be toxic. Traditional small-molecule cancer therapeutics such as doxorubicin, gemcitabine, fluorouracil, cisplatin, and paclitaxel suffer from limited selectivity between tumor and healthy tissues, leading to considerable side effects. Compared with the free drugs, nanoparticulate formulations frequently exert less systemic toxicity owing to their reduced volumes of distribution, which decreases drug access to critical organs such as the heart and kidney. In nanoparticulate form, the drug does not pass as easily through fenestrations in the blood vessels of those organs. Nanoparticles can also increase the deposition of drugs in tumors owing to the enhanced permeability and retention (EPR) effect, which takes advantage of leaky tumor microvasculature and a lack of developed lymphatic draining system.[7] It has also been demonstrated that nanoparticles are able to overcome biological barriers in the case of multidrug resistance, a phenomena in which small-molecule drugs are pumped out of cancer cell membranes by protein efflux pumps after patients have undergone multiple rounds of chemotherapy.[8]

Often, nanoparticle formulations are designed to solve simple problems. When hydrophobic drugs cannot be dissolved in water, nanoparticulate formulations are considered because the alternative is to dissolve the drugs with surfactants or nonaqueous solvents for administration.[9] Several successful

*Correspondence to: jflovell@buffalo.edu

[1]Department of Biomedical Engineering, University at Buffalo, State University of New York, Buffalo, NY, USA

[2]Department of Chemical and Biological Engineering, University at Buffalo, State University of New York, Buffalo, NY, USA

Conflict of interest: The authors have declared no conflicts of interest for this article.

BOX 1

MITIGATING TOXICITY OF EXISTING CHEMOTHERAPIES

One of the keys to the success of two well-known nanomedicines is their ability to reduce the toxicity of therapeutic agents. The liposomal doxorubicin formulation Doxil has been successful more for its ability to reduce the cardiotoxicity associated with the use of free doxorubicin than for its therapeutic effects. Cardiotoxicity is the main dose-limiting factor of doxorubicin and is significantly reduced by nanoparticle encapsulation. In this case, the nanoparticles serve to protect certain organs from accumulation of the chemotherapeutic agent. Abraxane, a nanoparticulate form of paclitaxel bound to human albumin, also demonstrates the ability of nanoparticles to reduce toxicity. In this case, the toxicity is not of the drug but of the excipients used to solubilize the drug. Paclitaxel is a poorly soluble hydrophobic drug with a propensity for aggregation and in order for it to be administered it needs to be solubilized with surfactants such as Cremophor EL. However, this surfactant can induce severe allergic reactions and limits the dosing of the paclitaxel. The development of Abraxane eliminates the toxicities associated with the delivery vehicle. In both cases, the use of nanoparticles serves to reduce adverse effects associated with an existing therapy.

nanomedicine formulations have become clinically relevant just by solving the problems of more straightforward drug formulations (Box 1). Introduction of a nanoparticulate system adds complexity that makes reproducible drug formulation and also safety regulation more difficult.[10] Thus, a strong advantage compared with existing standards of care should be apparent for new nanomedicines to hope to make it to the clinic. This is especially the case for the more exotic and complex types of nanomedicines that are in preclinical evaluation.[11]

NANOPARTICLE DESIGN STRATEGIES

General nanoparticle design principles are useful to consider for nanomedicines. Nanoparticle size, shape, surface chemistry, and composition are all key criteria that influence nanosystem behavior in biological contexts.[12] As shown in Figure 1, these numerous variables lead to a virtually endless combination of potential nanoparticles that could be developed and therefore a semirational approach is required.

Size affects the behavior of nanoparticles in the body (Figure 2). It has been shown that nanoparticles smaller than 30 nm can move into systemic circulation following administration into the lungs.[13] After nanoscale materials enter circulation in the blood stream, if they are small enough to pass through the glomerular basement membranes within the kidney, they will leave circulation through renal clearance into urine. Administration of quantum dots with hydrodynamic diameters of less than 5.5 nm resulted in rapid and efficient renal clearance.[14] Renal clearance of nanoparticles is attractive because the introduced nanomaterials enter and leave the body, mitigating many long-term safety concerns. However, renal clearance typically occurs too rapidly to enable enough accumulation of the nanoparticles into target tissues and therefore most administered nanomedicines avoid this effect. On the other hand, if nanoparticles are too large, they are also rapidly cleared from circulation. As blood passes through interendothelial cell slits of the spleen, nanoparticles that are over 200 nm in diameter get trapped and are rapidly cleared from circulation.[15] This is clearly affected by the deformability of the materials, because 8-μm-sized red blood cells pass through these slits constantly and remain in circulation for months.

For nanoparticles to be uptaken into solid tumors, size is also a critical factor. However, there can be variability depending on the type of tumor and because most studies have been restricted to animal models, there is less certainty on the topic. Following extravasation from blood vessels, it has been shown that nanoparticles that are less than 60 nm in diameter can better navigate the collagen-containing extracellular matrix and more deeply penetrate the bulk of the tumor.[16] It has also been shown that compared with larger ones, gold nanoparticles of 20 nm diameter can more easily diffuse out of tumors and are less likely to be retained.[17]

While the size of nanoparticles has been examined extensively, there has been somewhat less emphasis on their shape until recently. This is because for the most part, traditional nanoparticles used *in vivo* such as liposomes, polymeric nanoparticles, and proteins are roughly spherical in shape. The hydrodynamic radius, which is a standard measure of nanoparticle size, presumes this. However, several nonspherical nanoparticles such as gold nanorods and carbon nanotubes (CNTs) as well as other structures with high aspect ratios are now being investigated. For example, PEGylated filomicelles, which are long worm-like micelles, circulated for 1 week after intravenous injection in rodents, which is a much longer than typical spherical particles.[18]

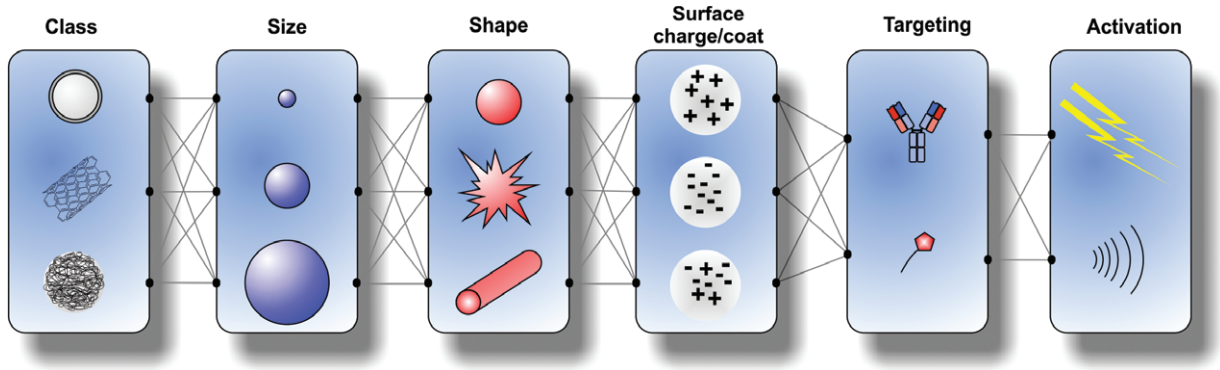

FIGURE 1 | Combinatorial criteria to be considered when designing nanoparticles. These include the class of nanoparticle size, shape, surface charge, targeting, and activation mechanism. Each factor affects the efficiency. Class determines the basic properties of the particle. Size, shape, surface charge, and targeting generally affect pharmacokinetics and biodistribution. Activation can provide improved distribution or bioavailability of encapsulated drugs.

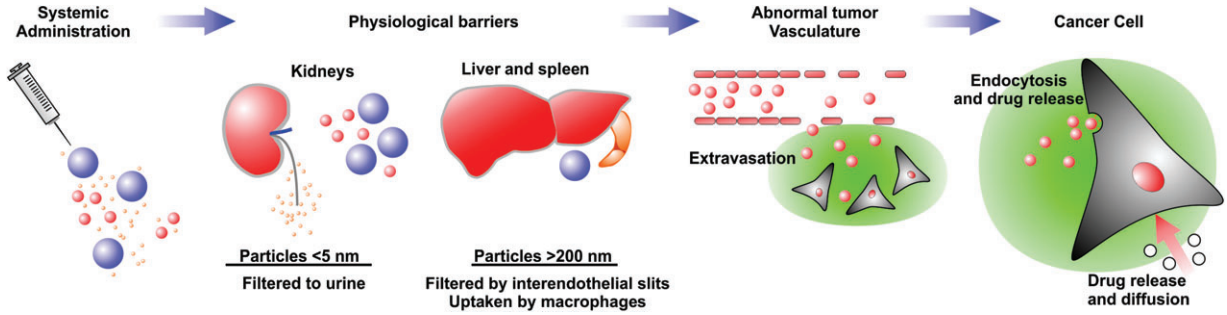

FIGURE 2 | *In vivo* fate of nanoparticles following systemic administration. Small nanoparticles can be cleared by the kidneys, whereas larger nanoparticles can be cleared by the liver and spleen. Nanoparticles then extravasate into the tumor tissue owing to the large fenestrations in the tumor vasculature. Extravasated nanoparticles deliver drugs to target cells through endocytosis or through the breakdown of the nanoparticles and release of the drug.

They could also be used for delivery of paclitaxel to tumors. Quasi-one-dimensional single-wall carbon nanotubes (SWNTs) have shown high tumor accumulation, with their nanoscale shape and flexibility likely contributing.[19] Magnetic iron oxide nanoworms also demonstrated that unique shapes and structures can result in improved *in vivo* behavior.[20]

In general, forming nanoparticles into complex shapes can be technically challenging. New advances in top-down nanofabrication using particle replication in nonwetting templates have opened the door for opportunities to explore nanoparticles with shapes that otherwise would be difficult to create.[21] Another approach makes use of viruses from nature, which exhibit a wonderful diversity of shapes and there have been efforts to use viral nanoparticles both as nanocarriers as well as templates for assembling other nanoparticles.[22] DNA nanotechnology is another area with great potential for designing nanoparticulate shapes of virtually any desired form using a bottom-up self-assembly approach[23] and these are now increasingly being used for *in vivo* experimentation.[24] Figure 3 shows some of the exotic shapes that are possible for nanomedicine. Based on the recent progress in developing biocompatible nanoparticles with more complex shapes, the relationships between nanoparticle shape and *in vivo* behaviors will be more clearly elucidated in coming years.

Surface charge is another important factor that should be considered when designing nanoparticles. Because the luminal surface of vascular endothelium is negatively charged, positively charged molecules tend to have a higher transvascular transport efficiency compared with neutral or negatively charged molecules of similar sizes.[25] This suggests that using cationic surface charges may enhance the delivery of nanoparticles in the blood vessels of the target tissues. However, positively charged molecules will bind nonspecifically to all blood vessel walls and therefore have a faster clearance rate compared with neutral or negatively charged molecules, which may counterbalance delivery advantages to target tissues.[26] Furthermore, cationic lipids and polymers, which can be easily used to confer a cationic nature

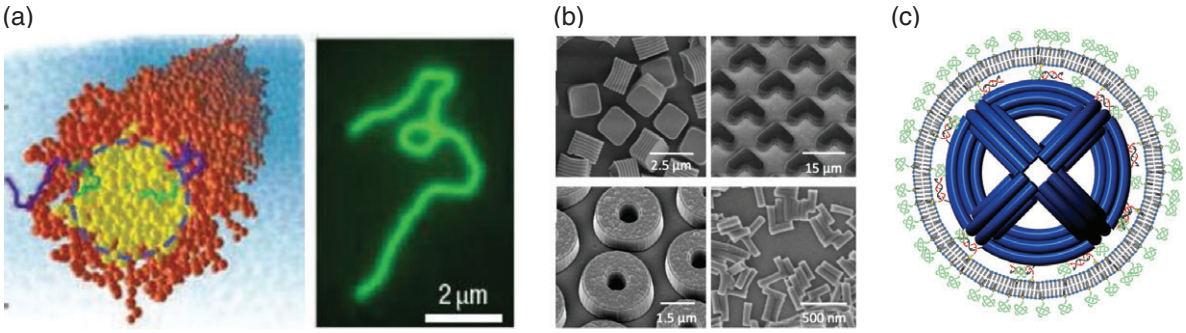

FIGURE 3 | (a) Filomicelles, self-assembled diblock copolymers; yellow/green indicates hydrophobic polymer center, orange/blue indicates hydrophilic polymer (left), fluorescence imaging of a single filomicelle showing its long size. (b) Scanning electron microscopic (SEM) images of particles produced by PRINT technology; cubic particles, hydrogel boomerangs, hydrogel toroids, and hydrogel rods. (c) Schematic of liposome-enclosed DNA nano-octahedron (DNO). The liposomes are fused to the DNO through DNA lipid complexes that bind the liposome bilayer to the DNO. This system uses the PEGylated liposomes to function as a viral-like capsid shell to protect the nanoparticle. (Reprinted with permission from Ref 18. Copyright 2007 Macmillan Publishers; Ref 21. Copyright 2011 John Wiley and Sons; and Ref 24. Copyright 2014 American Chemical Society)

upon nanoparticles of interest, pose concerns with respect to their safety.[27] It has been shown that strong positive or negative charges lead to rapid clearance of nanoparticles, whereas a slight negative charges lead to a lesser amount of phagocytosis.[28] Recently, zwitterionic charge coatings, which contain a mix of both positive and negative ionic charges, have been shown to improve nanoparticle circulation times.[29,30]

Upon administration into the blood stream, nanoparticles immediately become coated with a protein corona during the opsonization process. The surface chemistry of the nanoparticle plays a critical role in determining the corona composition, which can rapidly flag the nanoparticles for removal.[31] Coating nanoparticles with a protective layer of hydrophilic polyethylene glycol (PEG) has become standard practice for modifying surface chemistry.[32] PEG coatings for nanoparticle surface modification are widely commercially available, may improve the solubility of the nanoparticles, and in many cases have been shown to provide a degree of protection from rapid liver clearance by the reticular endothelial system.[33] However, there has been some controversy about mild immunogenic properties of the synthetic polymer.[34] Functionally, PEGylation does not confer circulation time to nanoparticles anywhere close to that of many native blood components. Alternative approaches, such as coating nanoparticles with red blood cell membranes,

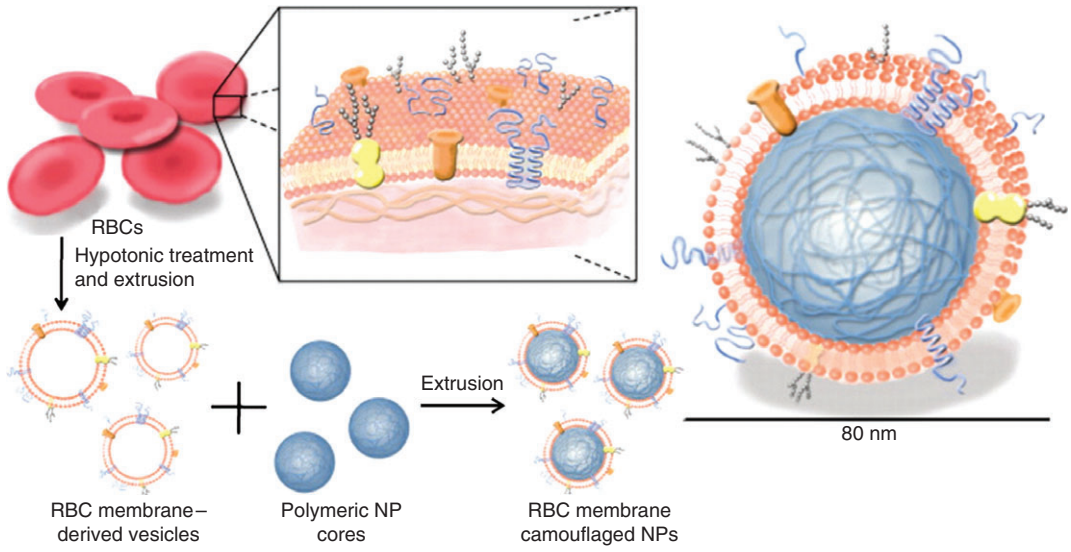

FIGURE 4 | Red blood cell (RBC)-membrane-coated polymeric nanoparticles. RBC membranes are isolated from the intracellular contents. The isolated RBC membranes are then fused to the polymeric nanoparticles with the aim of creating particles with increased circulation time. (Reprinted with permission from Ref 35. Copyright 2008 National Academy of Sciences)

have been proposed.[35] Figure 4 shows an illustrative schematic diagram of such a process.

For drug nanocarriers, an additional consideration is that drugs must be made bioavailable in the target tissues. If the drug is immediately released from the nanocarrier following administration, the nanocarrier serves only as a solubilizing vehicle, which in some cases is sufficient. If the drug is released too slowly from the nanocarrier it may not be able to reach its molecular targets to exert any effect. Thus, an appropriate and defined release mechanism is desired. Biocompatible polymeric nanoparticles have been extensively developed for that purpose and can tune the release rate of drugs from the carrier with exquisite control.[36] Other triggered release mechanisms can be achieved through designing nanoparticles that are responsive to certain environmental conditions such as pH, or through external stimuli such as heat.

The perfect nanoparticle would be able to target diseased locations without accumulating in healthy tissue. In practice, nanomedicines are far from this ideal. Similar to free drugs, nanoparticles that are developed to deliver drugs will induce side effects owing to drug accumulation in healthy organs. Removal of the particles from the blood owing to opsonization and uptake by the reticuloendothelial system (RES) is a major barrier.[37] Additionally, the serum proteins that adsorb to nanoparticles when they are introduced in the body may significantly interfere with targeting or triggered release approaches.

Another challenge is bioavailability. That is in this case having the drug in a form that can be toxic to the target cells. While nanoparticles can accumulate with large drug doses in some tumors, the drug often remains within the nanoparticles in the extracellular space outside of the cells. This is a challenge that may be overcome by a triggering mechanism that can release the drug from the nanoparticle at the target site or by engineering the nanoparticles to be more efficiently uptaken by cells. It is these barriers that many researchers aim to overcome when designing nanoparticles.

TARGETING MECHANISMS

Passive tumor targeting based on the EPR effect takes advantage of the abnormal vasculature and lack of lymphatic system in tumor tissue. Vessels in tumors are irregularly shaped and leaky owing to the rapid growth of tumors, resulting in abnormal blood supply. In tumor vasculature, the size of the gap between leaky endothelial cells is in the range of 100–780 nm depending on the type of tumor[38] as opposed to 5–10 nm in healthy vasculature.[39] It appears likely that even with improvements, passive targeting will still only achieve a relatively modest proportion of drug deposition into tumor tissues, with most of the drug being taken up by other organs. While improved passive targeting strategies may greatly enhance drug efficacy, the concept of a 'homing missile' that can specifically deliver nanoparticulate drug payloads to target sites remains a highly appealing yet elusive goal.

Active targeting involves functionalization of the surface of nanoparticles with receptor-specific agents such as small ligands, aptamers, peptides, and antibodies.[40] Ideally, the molecular target should be overexpressed in the tissue of interest and minimally expressed in nontargeted tissue as well as possess a rapid internalization rate.[41] Active targeting strategies were initially expected to deposit more drugs in the targeted tissues and reduce off-target effects. A few targeted therapeutic agents have been clinically approved, with many currently undergoing clinical trials. Table 1 gives some examples of targeted therapeutic agents. However, numerous studies have shown that active nanoparticulate targeting strategies fail to dramatically increase the concentration of drugs in tumors and, in many cases, the biodistribution of the therapeutic is barely changed.[42,43] This growing evidence suggests that the main factor that determines the accumulation of drugs in tumors is the EPR effect. However, it is important to note that in a number of cases active targeting has shown enhanced antitumor efficacy, including drug-loaded immunoliposomes.[44] The mechanism for this enhanced efficacy often stems from altered and improved cellular internalization. Despite the challenges of active targeting, it is worth describing several commonly used active targeting strategies owing to their great potential.

Integrins, which comprise a large family of membrane-bound dimer proteins, are expressed on the blood vessels of tissues affected by vascular disorders, angiogenesis, wounds, and other conditions.[45,46] The tripeptide motif of arginine–glycine–aspartic acid (RGD) binds to $\alpha v\beta 3$-integrins that are expressed in newly forming vasculature and has been used extensively in targeting applications. For example, drug-loaded, RGD-conjugated polymers and liposomes have been shown to target tumor angiogenic vasculature with higher biodistribution and efficacy than the untargeted versions.[47,48]

Epidermal growth factor receptors (EGFR) are transmembrane proteins that are expressed in many cancers and promote solid tumor growth.[49] EGFR is a family of receptors that comprises four members including EGFR, human EGFR-2 (HER-2), HER-3, and HER-4. HER-2, the second

TABLE 1 | Some Examples of Targeted Therapeutic Agents

Name	Nanoplatform/Agent	Status	Indications
Aurimune (CYT-6091)	Colloidal gold nanoparticles/recombinant human tumor necrosis factor alpha (rhTNF)	Phase II	Solid tumors
BIND-014	Prostate-specific membrane antigen (PSMA) PSMA-targeted polymer nanoparticle containing docetaxel	Phase II	Non-small cell lung cancer, prostate cancer
Cyclosert (CALAA-01)	Cyclodextrin siRNA	Phase Ib	Solid tumors
MBP-426	Transferrin-targeted oxaliplatin	Phase IIa	Gastric and esophageal adenocarcinoma
Mylotarg	Anti-CD33–calicheamicin conjugate	Approved then withdrawn	Refractory acute myelogenous leukemia
Ontak	Interleukin 2-targeted diphtheria toxin fragment	Approved in 2008	Cutaneous T-cell lymphoma (CTCL)
Rexin-G	Targeting retroviral vector microRNA-122	Phase III (USA)	Sarcoma, osteosarcoma, pancreatic cancer, and other solid tumor
SGT-53	Transferrin-targeted liposomes for p53 gene therapy	Phase I	Various cancers
CRLX101 (IT-101)	Cyclodextrin camptothecin formulation	Phase II	Various cancers

member of the EGFR family, has been extensively researched for antibody-targeting drug delivery. For instance, immunoliposomes were developed combining anti-HER2 monoclonal antibodies (mAbs) with the pharmacokinetics of sterically stabilized liposomes.[44] The enhanced antitumor efficacy primarily occurred through improved cellular internalization, as opposed to better overall tumor biodistribution.

Transferrin, an iron-carrying blood plasma glycoprotein, has been conjugated to nanoparticles to enhance cellular internalization via endocytosis.[50] The transferrin receptor is expressed in many types of tumors. For instance, CALAA-01 is a cyclodextrin-based, transferrin-targeted nanoparticle that has progressed to human clinical trials for siRNA delivery.[51] Similar to EGFR targeting, administration of transferrin-targeted gold nanoparticles showed that the targeting agent does not change nanoparticle biodistribution, but enhances intracellular delivery.[52]

Folate receptors are overexpressed in approximately 40% of human cancers and are one of the most intensively investigated targeting ligands.[53] Folate is an important metabolite for nucleobase synthesis. Conjugation of carboxylic acid of folic acid does not prevent recognition by the folate receptor, which has enabled many different targeting approaches. Folate-targeted, doxorubicin-loaded polymeric micelles were shown to be more effective than untargeted ones and also modestly enhanced tumor biodistribution.[54] The 60 nm paclitaxel and folate-conjugated nanoparticles did not deliver significantly more drug to tumors but efficacy was enhanced compared with nontargeted nanoparticles.[55]

NANOCARRIERS

Research in developing nanoparticles as delivery vehicles and imaging agents is ever increasing. Among the most commonly used nanoparticles are liposomes, polymer–drug conjugates, polymeric nanoparticles, micelles, CNTs, and quantum dots. Some nanoparticles have been successfully clinically applied and many more are currently being clinically evaluated. Table 2 lists representative nanomedicines that are currently approved. Table 3 lists some representative nanomedicines currently in clinical trials. Note that monoclonal antibodies are not included in these lists, although they could be considered to be a nanomedicine based on their size. It is likely that next-generation nanoparticles will build upon the existing foundation of current generation of nanoparticles that have progressed to the clinic.

Liposomes

Liposomes are commonly used as nanomedicines owing to their biocompatibility from being formed from lipids that are already found in the body. They are spherical vesicles composed of lipid bilayers which surround an aqueous core. They were first described by British hematologist Alec D Bangham in 1965.[56] Liposomes can be used as carriers for administration of pharmaceutical drugs, with the hydrophilic drugs encapsulated in the aqueous core and hydrophobic drugs retained within the bilayers. As of 2012, 12 liposomal drugs have been approved and many more are in clinical trials. The first generation of liposomes had short circulation time owing

TABLE 2 | Some Examples of Nanomedicines on the Market

Name	Nanomedicine	Status	Indications
Abraxane	Albumin-bound paclitaxel nanoparticles	Approved in 2005	Metastatic breast and pancreatic cancer
Ambisome	Liposomal amphotericin B	Approved in 1997	Fungal infections
Daunoxome	Liposomal daunorubicin	Approved in 1996	Kaposi's sarcoma
DepoDur	Liposomal morphine	Approved in 2004	Postsurgical pain relief
Doxil	Liposomal doxorubicin	Approved in 1995	Ovarian cancer, Kaposi's sarcoma
Genexol-PM	Polymeric micelles with paclitaxel	Available in Asian countries	Breast and lung cancers
Myocet	Liposomal doxorubicin (no PEGylation)	Available in Canada and Europe	Metastatic breast cancer
Neulasta	PEG–granulocyte colony-stimulating factor (PEG-CSF)	Approved in 2002	Febrile neutropenia
Oncaspar	PEG–L-asparaginase	Approved in 1994	Lymphocytic leukemia, non-Hodgkin's lymphoma
PEGASYS	PEG–interferon α2a	Approved in 2002	Hepatitis C
PEGIntron	PEG–interferon α2b	Approved in 2001	Hepatitis C
Visudyne	Liposomal verteporfin	Approved in 2000	Age-related macular degeneration

PEG, polyethylene glycol.

TABLE 3 | Some Examples of Nanomedicines Undergoing Clinical Trials

Product/Agent	Nanoplatform/Agent	Status	Indications
Combidex	Iron oxide nanoparticles	Phase III	Tumor imaging
CPX-1	Liposomal irinotecan:floxuridine	Phase II	Colorectal cancer
CPX-351	Liposomal cytarabine and daunorubicin	Phase III	Acute myeloid leukemia
S-CKD602	PEGylated liposomal belotecan	Phase II	Various cancers
SP1049C	P-glycoprotein targeting pluronic (poloxamer) micelle formulation of doxorubicin	Phase III	Various cancers
VivaGel (SPL7013)	Lysine-based dendrimer	Phase III	Topical microbicide for prevention of HIV and HSV
ThermoDox	Heat-triggered liposomal Dox	Phase III	Breast cancer, primary liver cancer
LiPlaCis	PLA2-triggered liposomal cisplatin	Phase I stopped	Various cancers

PLA2, phospholipase A2.

to rapid clearance by the RES. RES is a part of the immune system and consists of phagocytic cells such as monocytes and macrophages located in liver, spleen, lymph nodes, and bone marrow.[57] Incorporation of lipid-anchored PEG derivatives prolongs the circulating half-life of liposomes.[58] PEGylated liposomes, also named 'stealth liposomes', can reduce the uptake by RES because the long hydrophilic PEG chains act as a steric brush to suppress the clearance by RES. Liposomes have been examined in many different ways, including assessing the effects of size, dose, and surface charge on pharmacokinetic parameters and antitumor efficacy.[59] In addition to liposomes, lipid-based micelle-like nanoparticles are viable carriers for therapeutic and imaging agents.[60,61] One interesting approach is to use or mimic naturally occurring lipoprotein nanoparticles for anticancer applications.[62]

Doxil, approved in 1995, was one of the first nanodrugs approved by the United States Food and Drug Administration (FDA) for the treatment of HIV-related Kaposi's sarcoma and was subsequently approved for the treatment of platinum-resistant ovarian cancer and multiple myeloma. Doxil exemplifies long-circulating and stable PEGylated liposomes that use active loading driven by a transmembrane ammonium sulfate gradient to stably incorporate the doxorubicin (loading efficacy higher than 90%).[63] AmBisome is a unilamellar liposomal amphotericin B preparation for the systemic

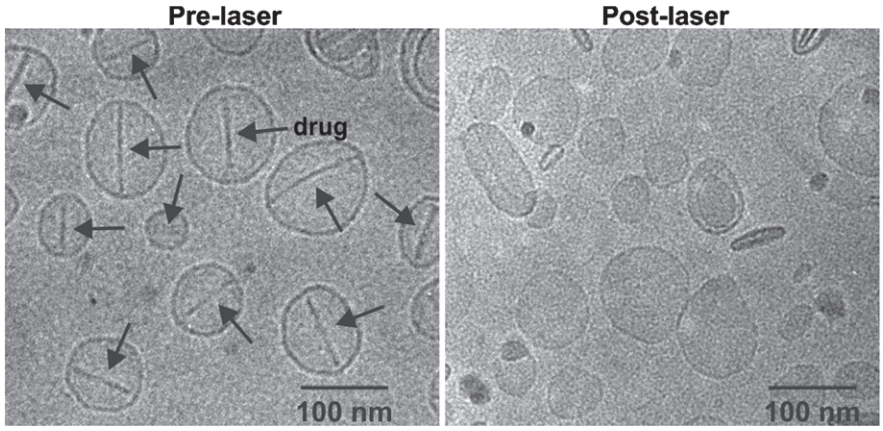

FIGURE 5 | Cryo-transmission microscopy images of Dox–PoP-liposomes before and after light irradiation. Arrows indicate the presence of doxorubicin sulfate crystals. While the crystals are present in the 'before' images they are not in the after images, indicating dissolution of the crystals and release of the drug under light irradiation with minimal effect on the morphology of the nanoparticles. (Reprinted with permission from Ref 76. Copyright 2014 Macmillan Publishers)

treatment of fungal infections.[64] The liposomal formulation has prolonged circulation time after intravenous administration. AmBisome effectively reduced amphotericin B-associated nephrotoxicity without loss in efficacy.[65] DepoDur, approved by FDA in 2004, is an extended release multivesicular liposomal epidural morphine. The main advantage of DepoDur is its extended release property, which reduces the frequency of dosing and more consistent serum concentrations. DepoDur has been increasingly used for treating acute postoperative pain without the use of infusions.[66]

A novel class of liposomes called porphysomes comprising of lipids that are made up of a single fatty acid side chain and a porphyrin group is recently developed.[67] Porphyrins have a long history of use as theranostic agents.[68] These liposomes can be assembled using different lipid compositions and tailored to different purposes. Among these uses are photothermal therapy (PTT),[69] photodynamic therapy (PDT),[70] and biophotonic imaging.[71] Porphysomes have been shown to be effective at curing tumors as a PTT agent where the intact nanoparticles are used to generate heat. In contrast, they are also used as PDT agents in which the dissociated porphyrins are used to generate singlet oxygen. The ability of porphyrins to chelate various metals makes them suitable for use as contrast agents for magnetic resonance[72] and radionuclide imaging.[73,74] They have also been shown to be excellent photoacoustic imaging contrast agents[71] owing to their high near-infrared range (NIR) absorption and high fluorescence quenching that results in conversion of absorbed light into heat, which is required to generate photoacoustic signals. It has also been shown that porphyrin-lipid-containing liposomes can entrap anticancer drugs and release them upon exposure to NIR irradiation.[75] This is shown in Figure 5.

Polymer-Conjugated Drugs

Polymer–drug conjugates form a well-established and clinically successful class of nanomedicine. PEG, *N*-(2-hydroxypropyl)methacrylamide (HPMA), polyglutamate (PGA), and dextrans are among the most frequently used hydrophilic polymers. Therapeutic proteins and small drugs can be conjugated with the hydrophilic polymers to increase circulation time, reduce immunogenicity, and enhance the therapeutic efficacy of the original drug. Polymer–drug conjugate also have increased drug deposition in tumor compared with free drug owing to the EPR effect. Currently, there are several polymer–drug conjugates approved by FDA, especially protein therapeutics including PEG–asparaginase,[77] PEG–interferon α2a,[78] PEG–interferon α2b,[79] and PEG–granulocyte colony-stimulating factor (PEG-CSF).[80]

HPMA has been extensively studied. Typically, HPMA copolymers can be designed to be biodegradable by conjugation with a short linker peptide that can be degraded by the lysosomal thiol-dependent protease cathepsin. Many HPMA copolymer–drug conjugates are in clinical trials including HPMA copolymer–doxorubicin,[81–83] HPMA copolymer–doxorubicin–galactosamine,[84] and HPMA copolymer–paclitaxel.[85] Among them, HPMA copolymer–doxorubicin–galactosamine is notable because it contains galactosamine that can promote liver targeting, through binding to the hepatic asialoglycoprotein receptor, which is highly expressed in normal hepatocytes. This is useful as targeting

specific diseased organs could allow for the reduction of cytotoxicity to other organs and healthy tissue. However, in early clinical trials while targeting to the liver was observed, significant preferential uptake in the tumor was not. PGA and dextran conjugates such as PGA–paclitaxel,[86] PGA–camptothecin,[87] and dextran–doxorubicin[88] have entered clinical trials.

Polymeric Nanoparticles

Polymeric nanoparticles are developed by polymers encapsulating drugs into the polymer matrix. The most frequently used polymers used to formulate polymeric nanoparticles are poly(lactic-*co*-glycolic acid) (PLGA), polylactides (PLA), and polycaprolactone (PCL) owing to their biodegradability and biocompatibility. The release rate can be tuned from days to months by changing the ratio between lactide and glycolide. Several polymeric nanoparticles are on the market. Genexol-PM is a polymeric formulation of paclitaxel.[89,90] Paclitaxel is effective for a wide range of cancers. However, owing to its hydrophobicity, paclitaxel requires the use of solubilizing agents such as Cremophor EL, which can cause serious hypersensitivity reactions and compromises the therapeutic value of paclitaxel. Polymeric paclitaxel addressed this issue and was approved in Asia for the treatment of breast and lung cancers. Considering that polypeptides are polymers, Abraxane could be considered another polymeric paclitaxel formulation approved by FDA for the treatment of breast cancer in 2005. It is a 130-nm, detergent-free and consists only of paclitaxel bound to human albumin, thus avoiding the toxicity and immunogenicity concerns of Cremophor. Binding between paclitaxel and albumin is noncovalent and reversible and allows for rapid release of the drug *in vivo*.[91]

BIND-014[92] is a PEGylated, PLGA-based targeted polymeric nanoparticle formulation of docetaxel. It has completed phase I clinical trial and recently entered phase II clinical trial for non-small cell lung cancer, prostate cancer, and metastatic castration-resistant prostate cancer. BIND-014 physically entraps docetaxel and is targeted to prostate-specific membrane antigen (PSMA) expressed on prostate cancer cells and the vasculature of most non-prostate solid tumors. Clinical trials have indicated that the BIND-014 is safe and has strong antitumor activity.

Pluronics are large, mass-produced triblock copolymers of polypropylene oxide and PEG that are relatively well defined. SP1049C is a pluronic micelle formulation of doxorubicin that targets P-glycoprotein. Doxorubicin is noncovalently incorporated into micelles with the pluronic block copolymers (Pluronic L61 and Pluronic F127). Pluronic L61 has been shown to enhance drug uptake in multidrug-resistant (MDR) cells with high expression of P-glycoprotein.[93,94] The depletion of ATP is significant as the mechanisms responsible for multidrug resistance are energy dependent.

A family of novel pluronic nanoparticles called nanonaps was recently developed that are self-assembled with extremely hydrophobic naphthlocyanines (Nc) dyes.[95] Unlike conventional micelles, nanonaps are kinetically stable and form frozen micelles that can be purified and concentrated to high dye concentrations. They withstood the harsh conditions in gastrointestinal (GI) tract and safely passed through it without systemic absorption, demonstrating that they can be used for safe GI imaging. It is likely that this approach can be applied for forming frozen drug micelles.

Dendrimers

Dendrimers are a relatively new class of polymeric materials. Different from the polymeric nanoparticles that are formed from linear polymers, dendrimers are highly branched macromolecules with a high degree of surface functionality and versatility. Dendrimers have well-defined chemical structure and can vary in size from 5 to 100 nm. Drugs can be covalently conjugated to the surface of the dendrimers or physically entrapped in the interior of the core.[96] Most dendrimers used for drug delivery are covalently conjugated with drugs to create a precisely defined nanomedicine, which is the fundamental advantage of dendrimers. Additionally, drugs that are physically entrapped into dendrimer cores can easily leak out when exposed to biological fluids before they reach the intended sites. Dendrimers can be conjugated with many different functional moieties such as imaging agents and targeting moieties in addition to drugs owing to their highly multivalent surface area.

The manufacturing process of dendrimers involves a series of repetitive steps starting with a central initiator core. Each growth step represents a new generation of polymer with a larger molecular diameter. Poly(amidoamine) (PAMAM), polypeptide, and polyester can be built into dendrimers.[97] Among them, PAMAM dendrimer is the most extensively investigated dendrimer. Surface-modified PAMAM dendrimers are generally nonimmunogenic, water soluble, and possess terminal modifiable amine functional groups.

VivaGel is a topical dendrimeric microbicide for prevention of HIV.[98] A phase I clinical trial demonstrated that VivaGel was generally safe and

well tolerated.[99] VivaGel contains a highly charged polyanionic surface to attach to targets on viruses, preventing virus attachment and/or absorption thus prevent infection.

Inorganic Nanoparticles

While most of the nanoparticles that have been translated to the clinic have been of organic nature, the field of nanotechnology has shown most interest in inorganic materials owing to their fascinating optical and physical properties. Many of these have been explored for use as nanomedicines with some examples of progression to early-stage clinical trials. Safety is a concern because many inorganic nanoparticles are formed from heavy metal ions with known toxicities and the particles may also be nondegradable and persistent in the body.[100]

Gold nanoparticles are versatile with a wide range of applications from use as delivery vectors, imaging agent, and photothermal therapeutic agents.[101] Gold nanoparticles comprise an inert gold core and a surface that is readily modified via sulfur–gold linkages. Depending on their shape, gold nanoparticles exhibit plasmon resonance that converts NIR light to heat, and can be engineered to remotely trigger drug release[102] and have been used for photothermal ablation of cancer.[103] Varying shape and size allow for tunable properties such as absorbance, which can be tailored to specific applications such as PTT for which the use of particles with absorbance in the NIR of the spectrum is desired.[104]

Gold nanoparticles have been used for targeted drug applications. Recombinant human tumor necrosis factor-α was bound to the surface of PEGylated colloidal gold particles. Preclinical tests showed rapid tumor accumulation following intravenous injection, with little accumulation in the liver and spleen, likely owing to the small size (27 nm) and RES-avoiding properties.[105] With promising preclinical results it progressed to clinical trials under the name Aurimune (CYT-6091).[106] Phase I clinical trial results indicated that CYT-6091 was well tolerated and show preferential uptake at the tumor site.[106] Currently, CYT-6091 is undergoing phase II clinical trials.

Iron oxide magnetic nanoparticles have excellent biocompatibility and have been approved for use as imaging agents. Iron oxide nanoparticles generally have a core–shell structure, an iron oxide core composed of magnetite or maghemite, a hydrophilic shell, usually composed of starch, polyvinyl alcohol, or dextrin. They typically exhibit superparamagnetism. Magnetic nanoparticles have been used as contrast agents for magnetic resonance imaging and as heat mediators for cancer hyperthermia therapy.[107] Magnetic iron oxide nanoworms are elongated, dextran-coated particles composed of a linear aggregate of 5–10 iron oxide nanoparticles (50–80 nm).[20] Different from the spherical-shaped nanoparticles that exhibit a high uptake by phagocytes, nanoworms with a linear shape revealed a lower uptake by phagocytes and have long circulating half-lives of 18 h. The elongated structure of the nanoworms also enhances the net magnetization and magnetic resonance contrast.

Quantum dots are nanometer-sized fluorescent semiconductor nanocrystals and can be modified to be water soluble and biocompatible.[108] Quantum dots are well known for their wide range of excitation spectra and narrow, symmetrical, and tunable emission spectra.[109] They have been extensively studied for bioimaging owing to their superior brightness and photostability compared with organic dyes.[110] They can be conjugated with many biological targets including antibodies, proteins, and nucleic acids for immunohistochemistry and *in situ* hybridization.[111] It has been shown that RGD-labeled quantum dots can effectively be used for cancer imaging *in vivo*.[108]

CNTs are emerging as a unique drug delivery system. CNTs are members of the fullerene family. SWNTs and multiple-walled carbon nanotubes (MWNTs) are the two main types of CNTs. SWNTs are composed of a single-layer graphite sheet, whereas MWNTs possess several graphite concentric layers. Most SWNTs have a diameter of approximately 1 nm while the length can vary to several hundreds or thousands of nanometers.[15] For MWNTs, the diameter varies from 1.5 to 100 nm with a length that generally ranges from 1 to 50 μm. CNTs are insoluble in water but can be made to dissolve by covalent or noncovalent functionalization. Their hydrophobicity also enables simple drug loading via adsorption of drugs. CNTs demonstrate high tensile strength, excellent chemical and thermal stability, and electrical and optical properties, which make them intriguing nanomaterials for a wide range of applications including the use of CNTs as ion channel blockers,[112] nanovectors for the delivery of therapeutics,[113] and biosensors.[114] As CNTs are relativity new to biotechnology, much about them remains to be studied especially their long-term safety and biocompatibility.[76]

STIMULATED DRUG RELEASE

There are at least two types of stimulated drug release: environmentally triggered release and externally triggered release. Environmental release occurs when local stimuli, such as pH, cause the nanoparticles to release their contents. Externally triggered release

occurs when an external stimulus, such as applied heat or light, induces release of entrapped contents. Both concepts allow for the release of drug at a target site, the main difference being that external release mechanisms offer more control, for on-demand release. However, external release mechanisms also are significantly more difficult to implement are limited to treating localized conditions such as a problematic primary tumor as opposed to the metastatic disease.

pH Triggering

pH-triggered release can be subdivided into three categories: orally deliverable drugs, tissue-level mechanisms, and cellular-level mechanisms.[115] In the case of orally delivered drugs, the goal is often to encapsulate the drugs so they pass through the acidic conditions of the stomach without degradation, and then release into the higher pH environments of the duodenum, and other parts of the GI tract. In this case, drug release is achieved by pH-dependent swelling, dissolution, or changes in surface charge.[116] One potential application for this system is through oral administration of insulin-loaded nanoparticles. Orally administered insulin-loaded polymer-based nanoparticles have been shown to be able to protect their contents through the stomach and deliver them into the intestines.[117] However, getting the insulin from the intestines into the blood stream remains a challenge as enzymes in the intestines will degrade the nanoparticles and insulin as well. Additionally, the doses required to reduce blood glucose levels for orally administered insulin are significantly higher than required for injected insulin (30–100 vs 1 IU/kg).[118]

Tissue-level mechanisms are related to the Warburg effect, whereby the tumor environment exhibits a pH value of 0.5–1 lower than physiologically normal tissues.[119,120] Because of hypoxic conditions, tumor cells switch to anaerobic respiration and generate excessive lactic acid, which causes the more acidic conditions. Nanoparticles are engineered to become destabilized and release their drug content at this reduced pH.[115,120,121] This may be achieved by designing nanoparticles such as polymeric micelles that dissociate under the mild acidic conditions of the tumor environment,[121] or pH-induced swelling.[115]

In cellular-level mechanisms, release of the drug occurs after the nanoparticles have been uptaken by cells. Following endocytosis, the nanoparticles are subjected to an acidic pH environment of 5–6.5 in endosomes and 4–5 in lysosomes.[122] Drug release is achieved similarly to the other pH mechanisms with release being induced by swelling, dissolution, or acid-induced bond cleavage of the carrier as well as destabilization of the endosomal membranes.[120,115] pH-sensitive liposomes have also been developed extensively by the Szoka group that are activated during endocytic uptake based on changes in charge that occur in the acidifying endosomal and lysosomal environments.[123]

It is possible for multiple pH-targeting strategies to be used concurrently. TAT peptide-based micelles are an example. They are polymer-based micelles to which are connected a PEG-conjugated TAT complex. This complex at physiological pH (7.4) is shielded by formation of a complex with a copolymer of PEG and poly(methacryloyl sulfadimethoxine) (PSD). Under the mild acidic conditions of the tumor environment the PSD shielding complex dissociates leaving the TAT exposed.[124] TAT, a HIV-derived nonspecific cell-penetrating peptide, increases the uptake of the micelles through endocytosis. Following endocytosis, the micelles disintegrate in the low pH environment, releasing entrapped doxorubicin within the endosomes. This system has been shown to suppress tumor growth in mice.[125]

Enzymatic Triggering

Enzymatic degradable nanoparticles work by releasing their encapsulated contents when exposed to the enzymes found at the target site. These delivery systems can be designed to be responsive to many different enzymes. This approach has been applied extensively to functional imaging probes.[126,127] For example, nanoparticles made with peptide linkages may be degraded by proteases, whereas those made with phospholipids can be degraded by lipases.[128] This system has the potential to induce minimal activation while the nanoparticles are in circulation in the blood. However, as many enzymes can be found in both healthy and diseased cells, the use of enzymes must be complemented by a specific targeting strategy or the use of enzymes that are present at greater levels in the diseased cells.[120,128,129]

An example of the application of this mechanism is liposomes designed to be degraded by secretory phospholipase A2 (sPLA2). sPLA2 is a lipid-hydrolyzing enzyme that is prevalent in the extracellular space of tumors. The responsiveness of liposomes to sPLA2 can by adjusted by altering the lipid composition. Cisplatin-loaded sPLA2-responsive liposomes were shown to effectively suppress tumor growth in nude mouse xenographs.[130] Hydrophobic drugs may also be conjugated directly to the hydroxyl group normally occupied by the lipid fatty acid side chain. At the target tissue, lipases may then cleave and liberate the drug. Phospholipid-fused porphyrins,[131]

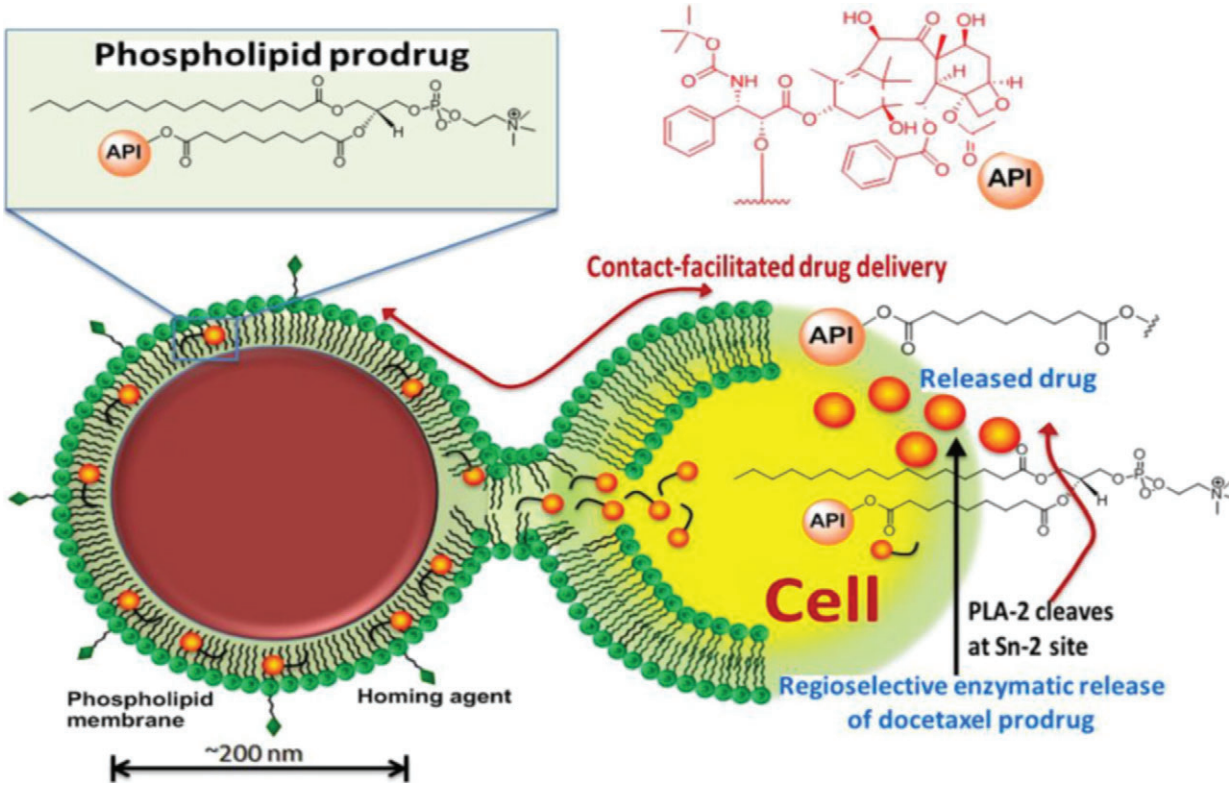

FIGURE 6 | Schematic of lipase-cleavable docetaxel prodrug concept. In this system, a lipophilic enzymatically cleavable prodrug is entrapped in the phospholipid layer of the nanoparticle. The nanoparticle is targeted to cells through contact-facilitated drug delivery where the phospholipid layer of the nanoparticle fuses with the cell membrane. The prodrug is then transferred into the cell where it undergoes enzymatic cleavage. (Reprinted with permission from Ref 133. Copyright 2014 Ivyspring)

mycotoxins,[132] and taxanes have all been assembled into nanoparticles for this lipase-activated mechanism.[133] This concept is illustrated in Figure 6, with a docetaxel–phospholipid prodrug.

Another family of enzymes that are linked to cancers is the matrix metalloproteinases (MMPs). These proteases degrade the extracellular matrix, thus enabling the spread of tumor cells. One interesting approach developed 100-nm nanoparticles that themselves contained smaller nanoparticles that could be released upon cleavage of the larger nanoparticle by MMP-2.[134] This concept is shown in Figure 7. In this manner, the larger nanoparticle can effectively accumulate in the tumor via the EPR effect and upon proteolytic cleavage the smaller nanoparticles are released and can deeply penetrate the tumor.

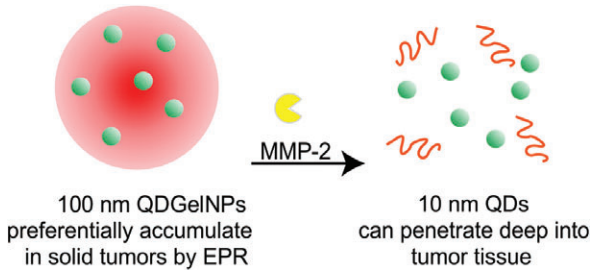

FIGURE 7 | Protease-activated drug delivery. Multistage quantum dot gelatin nanoparticles (QDGelNPs) experience a size reduction through cleavage of their gelatin scaffold by matrix metalloproteinase 2 (MMP-2), a protease that is highly expressed in tumors. (Reprinted with permission from Ref 134. Copyright 2011 National Academy of Sciences)

Heat Triggering

Heat-triggered release typically involves heating drug-encapsulated nanoparticles such as liposomes or polymer-based nanoparticles to a point at which entrapped drug becomes released. Generally, these involve the use of an external heat source to induce a change in the nanoparticle which makes them permeable.[135,136] In the case of liposomes, for example, heating above a critical transition temperature causes the liposome bilayer to change from a ridged crystalline phase to a more fluid liquid crystalline phase.[137] While this is often achieved by applying an external source of heat, other techniques have been developed, which uses alternative external

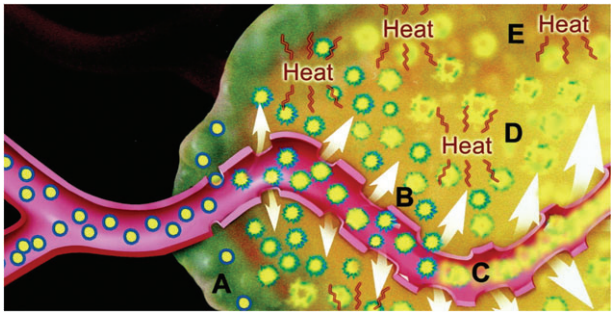

FIGURE 8 | Heat-triggered drug release. Specially designed Thermodox liposomes extravasate into the tumor through pores in leaky tumor blood vessels (a). Hyperthermia increases the blood vessel pore sizes (b). Hyperthermia triggers drug release from the liposomes in both the tumor blood vessels (c) and the tumor tissue (d). Hyperthermia itself can also be toxic to cancer cells (e). (Reprinted with permission from Ref 79. Copyright 2000 American Association for Cancer Research)

stimuli such as magnetic fields and light irradiation, along with entrapped nanoparticles capable of generating heat.[138,139] In order for these systems to be clinically applicable they need to meet two key requirements, first the drug should be released quickly upon application of the stimuli; second release should occur at temperature slightly above body temperature (39–40°C), which is considered to be mild hyperthermia because at more elevated temperatures vascular shutdown occurs.[140,141]

Of these techniques, the use of an external heat source with a special liposomal formulation, Thermodox, has been successful in advancing through phase III human clinical trials. Thermodox is a liposomal formulation of doxorubicin with rapid release of the drug under mild hyperthermia conditions. It is currently being clinically studied for the treatment of colorectal, breast, and liver cancers with one phase III clinical trial for primary liver cancer having been completed.[142,143] In addition to drug release from the liposomes at elevated temperatures, Thermodox seeks to take advantage of the therapeutic effects of hyperthermia itself. This includes increasing blood flow and tumor vessel permeability to nanoparticles.[141] In the clinical trials, the liposomes were combined with radiofrequency ablation therapy, which itself kills tumors by heating them to elevated temperatures. In these trials, the goal was for the liposomes to treat the cancer cells on the perimeter of the ablation zone where the temperature would be high enough to induce release but not sufficient to kill the cells on its own.[144] Figure 8 shows a schematic representation of the Thermodox activation mechanism.

Magnetic Triggering

Magnetic-triggered release can be achieved by two methods: through the use of heat-generating particles such as iron oxide or through mechanical mechanisms. Although a few mechanical mechanisms have been demonstrated these have not been as well studied as the heat-based mechanisms. A mechanism has been demonstrated in which release from nanospheres is induced by a high-frequency magnetic field which causes vibrations rupturing the shell of the particle.[139,145] The heat-triggered mechanisms involve entrapping heat-generating particles in a thermoresponsive nanoparticle. Upon the application of an external magnetic field the particles generate heat that induces drug release from the nanoparticles.[146–149] This system works similarly to the heat-triggered release system above except that the source of the heat is localized to the nanoparticles.

This has been demonstrated with magnetoliposomes, in which iron oxide particles were entrapped within liposomes and loaded with doxorubicin.[146] In this case, liposomes with a release temperature of 42°C were used and maximum drug release was achieved after 6 min. Heating of the bulk solution was minimal, though dependent on the concentrations used. While this demonstrates release can occur in the absence of significant heating, heating can also be beneficial. Heat-based treatments in which magnetic nanoparticles are used to induce hyperthermia are currently being clinically evaluated.[150]

Ultrasonic Triggering

Ultrasound has been shown to be able to release the contents from nanoparticles. This is achieved typically owing to cavitation induced under ultrasound irradiation,[151–154] though ultrasound heat-mediated release mechanisms also exist.[155] In this system, the ultrasound causes the formation of vapor bubbles that permeabilize the nanoparticle, allowing the entrapped drug to be released. One advantage of this system is that ultrasound is noninvasive; however, it can also cause cellular damage.[153,154]

This has been shown to be effective *in vivo* with cisplatin-loaded liposomes and low-frequency ultrasound (LFUS). In this study, a stealth formulation of cisplatin liposomes that have been shown to suffer from poor bioavailability due to slow release kinetics was used with LFUS to treat C26 tumors on the footpad of BALB/c mice. The results showed that the combination of the liposomes and LFUS improves the effectiveness of the liposomes owing to the increase of the bioavailability of the liposomes.[156]

Light Triggering

Many nanotechnology-based mechanisms involving light activation have been developed.[157]

Photochemical mechanisms and heat-related mechanisms are the two main categories. Photochemical mechanisms involve light-induced chemical reactions that lead to the permeabilization of the nanoparticles. These include reactions such as photooxidation, photoisomerization, and photocleavage. The heat-related mechanisms work similarly to the magnetic-triggered release in that light-sensitive heat-generating particles such as gold nanoparticles are entrapped within the nanoparticles. When the nanoparticles are treated with light, the heat-generating particles generate heat and cause release of the entrapped contents owing to thermally induced permeability. More novel methods for light-triggered release have also been shown. For example, the use of gold nanoparticle-tethered liposomes has been shown to release the contents through cavitation similar to the ultrasound mechanism,[102] and the use of channel proteins embedded within liposomes that open upon laser irradiation.[158]

For light-triggered release to be viable clinically, the wavelengths of light used would optimally be in the NIR of the spectrum as this is the most biologically compatible range. There are two primary reasons for this: first, NIR light provides better tissue penetration than ultraviolet (UV) light on the other end of the spectrum. Second, UV light poses phototoxicity to healthy tissue and, therefore, may not be safe. In addition, many photochemical mechanisms also tend to produce toxic reactive species, making them unlikely to be widely used. Methods that rely on photophysical mechanisms that are activated in the NIR range are appealing because they may not have as many potential phototoxicity risks.[138,159,160]

CONCLUSION

The unique properties of carefully designed nanomedicines hold potential for the treatment of diseases. The goal of nanomedical engineering is to develop nanoparticles that migrate to where they are intended to go and exert therapeutic effect there. This may be achieved by minimizing their removal from the body by physiological barriers and the immune system. Currently, the nanomedicines that have been clinically approved generally are formed from relatively simple, rather than complex formulations. However, the potential payoff of targeted and triggered delivery is high enough to warrant development of more advanced systems. In addition to innovating new and potentially revolutionary materials and approaches for nanomedicines, it is imperative for the success of the field that future works focus on determining how to improve quantitative therapeutic biodistribution and bioavailability to target tissues. Collective and quantitative data are required to better elucidate which strategies hold the most potential for further research investment. From a clinical perspective, it is expected that nanomedical engineering will bring an increasing number of unique treatments into early-stage clinical trials for evaluation with hopes of better disease treatments and outcomes.

ACKNOWLEDGMENTS

This work was made possible with support from the National Institutes of Health (R01EB017270 and DP5OD017898) and the Korean Ministry of Science, ICT and Future Planning (IT Consilience Creative Program, NIPA-2013-H0203-13-1001).

REFERENCES

1. Kim BYS, Rutka JT, Chan WCW. Nanomedicine. *N Engl J Med* 2010, 363:2434–2443.
2. Agasti SS, Rana S, Park M-H, Kim CK, You C-C, Rotello VM. Nanoparticles for detection and diagnosis. *Adv Drug Deliv Rev* 2010, 62:316–328.
3. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotechnol* 2007, 2: 751–760.
4. Mulder WJM, Jaffer FA, Fayad ZA, Nahrendorf M. Imaging and nanomedicine in inflammatory atherosclerosis. *Sci Transl Med* 2014, 6:239sr1.
5. Pison U, Welte T, Giersig M, Groneberg DA. Nanomedicine for respiratory diseases. *Eur J Pharmacol* 2006, 533:341–350.
6. Wagner V, Dullaart A, Bock A-K, Zweck A. The emerging nanomedicine landscape. *Nat Biotechnol* 2006, 24:1211–1217.
7. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J Controlled Release* 2000, 65:271–284.
8. Dong X, Mattingly CA, Tseng MT, Cho MJ, Liu Y, Adams VR, Mumper RJ. Doxorubicin and

paclitaxel-loaded lipid-based nanoparticles overcome multidrug resistance by inhibiting P-glycoprotein and depleting ATP. *Cancer Res* 2009, 69:3918–3926.

9. Strickley RG. Solubilizing excipients in oral and injectable formulations. *Pharm Res* 2004, 21:201–230.
10. Hamburg MA. FDA's approach to regulation of products of nanotechnology. *Science* 2012, 336:299–300.
11. Venditto VJ, Szoka FC Jr. Cancer nanomedicines: so many papers and so few drugs!. *Adv Drug Deliv Rev* 2013, 65:80–88.
12. Albanese A, Tang PS, Chan WCW. The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annu Rev Biomed Eng* 2012, 14:1–16.
13. Choi HS, Ashitate Y, Lee JH, Kim SH, Matsui A, Insin N, Bawendi MG, Semmler-Behnke M, Frangioni JV, Tsuda A. Rapid translocation of nanoparticles from the lung airspaces to the body. *Nat Biotechnol* 2010, 28:1300–1303.
14. Soo Choi H, Liu W, Misra P, Tanaka E, Zimmer JP, Itty Ipe B, Bawendi MG, Frangioni JV. Renal clearance of quantum dots. *Nat Biotechnol* 2007, 25:1165–1170.
15. Moghimi SM, Hunter AC, Murray JC. Nanomedicine: current status and future prospects. *FASEB J* 2005, 19:311–330.
16. Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. *Nat Rev Clin Oncol* 2010, 7:653–664.
17. Perrault SD, Walkey C, Jennings T, Fischer HC, Chan WCW. Mediating tumor targeting efficiency of nanoparticles through design. *Nano Lett* 2009, 9:1909–1915.
18. Geng Y, Dalhaimer P, Cai S, Tsai R, Tewari M, Minko T, Discher DE. Shape effects of filaments versus spherical particles in flow and drug delivery. *Nat Nanotechnol* 2007, 2:249–255.
19. Liu Z, Cai W, He L, Nakayama N, Chen K, Sun X, Chen X, Dai H. In vivo biodistribution and highly efficient tumour targeting of carbon nanotubes in mice. *Nat Nanotechnol* 2007, 2:47–52.
20. Park J-H, von Maltzahn G, Zhang L, Schwartz MP, Ruoslahti E, Bhatia SN, Sailor MJ. Magnetic iron oxide nanoworms for tumor targeting and imaging. *Adv Mater* 2008, 20:1630–1635.
21. Wang J, Byrne JD, Napier ME, DeSimone JM. More effective nanomedicines through particle design. *Small* 2011, 7:1919–1931.
22. Steinmetz NF. Viral nanoparticles as platforms for next-generation therapeutics and imaging devices. *Nanomed Nanotechnol Biol Med* 2010, 6:634–641.
23. Pinheiro AV, Han D, Shih WM, Yan H. Challenges and opportunities for structural DNA nanotechnology. *Nat Nanotechnol* 2011, 6:763–772.
24. Perrault SD, Shih WM. Virus-inspired membrane encapsulation of DNA nanostructures to achieve in vivo stability. *ACS Nano* 2014, 8:5132–5140.
25. Turner MR, Clough G, Michel CC. The effects of cationised ferritin and native ferritin upon the filtration coefficient of single frog capillaries. Evidence that proteins in the endothelial cell coat influence permeability. *Microvasc Res* 1983, 25:205–222.
26. Dellian M, Yuan F, Trubetskoy VS, Torchilin VP, Jain RK. Vascular permeability in a human tumour xenograft: molecular charge dependence. *Br J Cancer* 2000, 82:1513–1518.
27. Lv H, Zhang S, Wang B, Cui S, Yan J. Toxicity of cationic lipids and cationic polymers in gene delivery. *J Controlled Release* 2006, 114:100–109.
28. He C, Hu Y, Yin L, Tang C, Yin C. Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles. *Biomaterials* 2010, 31:3657–3666.
29. Liu X, Li H, Chen Y, Jin Q, Ren K, Ji J. Mixed-charge nanoparticles for long circulation, low reticuloendothelial system clearance, and high tumor accumulation. *Adv Healthc Mater* 2014, 3:1439–1447.
30. Xiao W, Lin J, Li M, Ma Y, Chen Y, Zhang C, Li D, Gu H. Prolonged in vivo circulation time by zwitterionic modification of magnetite nanoparticles for blood pool contrast agents. *Contrast Media Mol Imaging* 2012, 7:320–327.
31. Walkey CD, Chan WCW. Understanding and controlling the interaction of nanomaterials with proteins in a physiological environment. *Chem Soc Rev* 2012, 41:2780.
32. Harris JM, Chess RB. Effect of pegylation on pharmaceuticals. *Nat Rev Drug Discov* 2003, 2:214–221.
33. Owens DE III, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *Int J Pharm* 2006, 307:93–102.
34. Schellekens H, Hennink WE, Brinks V. The immunogenicity of polyethylene glycol: facts and fiction. *Pharm Res* 2013, 30:1729–1734.
35. Hu C-MJ, Zhang L, Aryal S, Cheung C, Fang RH, Zhang L. Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. *Proc Natl Acad Sci U S A* 2011, 108:10980–10985.
36. Hans ML, Lowman AM. Biodegradable nanoparticles for drug delivery and targeting. *Curr Opin Solid State Mater Sci* 2002, 6:319–327.
37. Moghimi SM, Hunter AC, Andresen TL. Factors controlling nanoparticle pharmacokinetics: an integrated analysis and perspective. *Annu Rev Pharmacol Toxicol* 2012, 52:481–503.
38. Hobbs SK, Monsky WL, Yuan F, Roberts WG, Griffith L, Torchilin VP, Jain RK. Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. *Proc Natl Acad Sci U S A* 1998, 95:4607–4612.

39. Haley B, Frenkel E. Nanoparticles for drug delivery in cancer treatment. *Urol Oncol Semin Orig Investig* 2008, 26:57–64.
40. Byrne JD, Betancourt T, Brannon-Peppas L. Active targeting schemes for nanoparticle systems in cancer therapeutics. *Adv Drug Deliv Rev* 2008, 60:1615–1626.
41. Allen TM. Ligand-targeted therapeutics in anticancer therapy. *Nat Rev Cancer* 2002, 2:750–763.
42. Kirpotin DB, Drummond DC, Shao Y, Shalaby MR, Hong K, Nielsen UB, Marks JD, Benz CC, Park JW. Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. *Cancer Res* 2006, 66:6732–6740.
43. Pirollo KF, Chang EH. Does a targeting ligand influence nanoparticle tumor localization or uptake? *Trends Biotechnol* 2008, 26:552–558.
44. Park JW, Hong K, Kirpotin DB, Colbern G, Shalaby R, Baselga J, Shao Y, Nielsen UB, Marks JD, Moore D, et al. Anti-HER2 immunoliposomes enhanced efficacy attributable to targeted delivery. *Clin Cancer Res* 2002, 8:1172–1181.
45. Niu G. Why integrin as a primary target for imaging and therapy. *Theranostics* 2011, 1:30–47.
46. Ruoslahti E. Rgd and other recognition sequences for integrins. *Annu Rev Cell Dev Biol* 1996, 12: 697–715.
47. Mitra A, Mulholland J, Nan A, McNeill E, Ghandehari H, Line BR. Targeting tumor angiogenic vasculature using polymer–RGD conjugates. *J Controlled Release* 2005, 102:191–201.
48. Dubey PK, Mishra V, Jain S, Mahor S, Vyas SP. Liposomes modified with cyclic RGD peptide for tumor targeting. *J Drug Target* 2004, 12:257–264.
49. Nicholson RI, Gee JMW, Harper ME. EGFR and cancer prognosis. *Eur J Cancer* 2001, 37(suppl 4):9–15.
50. Qian ZM, Li H, Sun H, Ho K. Targeted drug delivery via the transferrin receptor-mediated endocytosis pathway. *Pharmacol Rev* 2002, 54:561–587.
51. Davis ME, Zuckerman JE, Choi CHJ, Seligson D, Tolcher A, Alabi CA, Yen Y, Heidel JD, Ribas A. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature* 2010, 464:1067–1070.
52. Choi CHJ, Alabi CA, Webster P, Davis ME. Mechanism of active targeting in solid tumors with transferrin-containing gold nanoparticles. *Proc Natl Acad Sci U S A* 2010, 107:1235–1240.
53. Low PS, Kularatne SA. Folate-targeted therapeutic and imaging agents for cancer. *Curr Opin Chem Biol* 2009, 13:256–262.
54. Yoo HS, Park TG. Folate receptor targeted biodegradable polymeric doxorubicin micelles. *J Controlled Release* 2004, 96:273–283.
55. Wang X, Li J, Wang Y, Cho KJ, Kim G, Gjyrezi A, Koenig L, Giannakakou P, Shin HJ, Tighiouart M, et al. HFT-T, a targeting nanoparticle, enhances specific delivery of paclitaxel to folate receptor-positive tumors. *ACS Nano* 2009, 3:3165–3174.
56. Bangham AD, Horne RW. Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. *J Mol Biol* 1964, 8:660–668.
57. Nie S. Understanding and overcoming major barriers in cancer nanomedicine. *Nanomedicine* 2010, 5:523–528.
58. Klibanov AL, Maruyama K, Torchilin VP, Huang L. Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. *FEBS Lett* 1990, 268:235–237.
59. Drummond DC, Meyer O, Hong K, Kirpotin DB, Papahadjopoulos D. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. *Pharmacol Rev* 1999, 51:691–744.
60. Constantinides PP, Chaubal MV, Shorr R. Advances in lipid nanodispersions for parenteral drug delivery and targeting. *Adv Drug Deliv Rev* 2008, 60:757–767.
61. Mulder WJM, Strijkers GJ, van Tilborg GAF, Griffioen AW, Nicolay K. Lipid-based nanoparticles for contrast-enhanced MRI and molecular imaging. *NMR Biomed* 2006, 19:142–164.
62. Ng KK, Lovell JF, Zheng G. Lipoprotein-inspired nanoparticles for cancer theranostics. *Acc Chem Res* 2011, 44:1105–1113.
63. Barenholz Y. (Chezy). Doxil®—the first FDA-approved nano-drug: lessons learned. *J Controlled Release* 2012, 160:117–134.
64. Boswell GW, Buell D, Bekersky I. AmBisome (liposomal amphotericin B): a comparative review. *J Clin Pharmacol* 1998, 38:583–592.
65. Fanos V, Cataldi L. Amphotericin B-induced nephrotoxicity: a review. *J Chemother* 2000, 12:463–470.
66. Nagle PC, Gerancher JC. DepoDur® (extended-release epidural morphine): a review of an old drug in a new vehicle. *Tech Reg Anesth Pain Manage* 2007, 11:9–18.
67. Huynh E, Zheng G. Porphysome nanotechnology: a paradigm shift in lipid-based supramolecular structures. *Nano Today* 2014, 9:212–222.
68. Zhang Y, Lovell JF. Porphyrins as theranostic agents from prehistoric to modern times. *Theranostics* 2012, 2:905–915.
69. Jin CS, Lovell JF, Chen J, Zheng G. Ablation of hypoxic tumors with dose-equivalent photothermal, but not photodynamic, therapy using a nanostructured porphyrin assembly. *ACS Nano* 2013, 7:2541–2550.
70. Jin CS, Cui L, Wang F, Chen J, Zheng G. Targeting-triggered porphysome nanostructure disruption for activatable photodynamic therapy. *Adv Healthc Mater* 2014, 3:1240–1249.

71. Lovell JF, Jin CS, Huynh E, Jin H, Kim C, Rubinstein JL, Chan WCW, Cao W, Wang LV, Zheng G. Porphysome nanovesicles generated by porphyrin bilayers for use as multimodal biophotonic contrast agents. *Nat Mater* 2011, 10:324–332.
72. MacDonald TD, Liu TW, Zheng G. An MRI-sensitive, non-photobleachable porphysome photothermal agent. *Angew Chem Int Ed Engl* 2014, 53:6956–6959.
73. Liu TW, MacDonald TD, Jin CS, Gold JM, Bristow RG, Wilson BC, Zheng G. Inherently multimodal nanoparticle-driven tracking and real-time delineation of orthotopic prostate tumors and micrometastases. *ACS Nano* 2013, 7:4221–4232.
74. Lee J-H, Shao S, Cheng KT, Lovell JF, Paik CH. 99mTc-labeled porphyrin–lipid nanovesicles. *J Liposome Res* 2014:1–6. doi:10.3109/08982104.2014.932379.
75. Carter KA, Shao S, Hoopes MI, Luo D, Ahsan B, Grigoryants VM, Song W, Huang H, Zhang G, Pandey RK, et al. Porphyrin–phospholipid liposomes permeabilized by near-infrared light. *Nat Commun* 2014, 5:3546. doi: 10.1038/ncomms4546.
76. Volder MFLD, Tawfick SH, Baughman RH, Hart AJ. Carbon nanotubes: present and future commercial applications. *Science* 2013, 339:535–539.
77. Fu CH, Sakamoto KM. PEG-asparaginase. *Expert Opin Pharmacother* 2007, 8:1977–1984.
78. Rajender Reddy K, Modi MW, Pedder S. Use of peginterferon alfa-2a (40 KD) (Pegasys®) for the treatment of hepatitis C. *Adv Drug Deliv Rev* 2002, 54:571–586.
79. Wang Y-S, Youngster S, Grace M, Bausch J, Bordens R, Wyss DF. Structural and biological characterization of pegylated recombinant interferon α-2b and its therapeutic implications. *Adv Drug Deliv Rev* 2002, 54:547–570.
80. Molineux G. The design and development of pegfilgrastim (PEG-rmetHuG-CSF, Neulasta). *Curr Pharm Des* 2004, 10:1235–1244.
81. Satchi R, Connors TA, Duncan R. PDEPT: polymer-directed enzyme prodrug therapy. *Br J Cancer* 2001, 85:1070–1076.
82. Satchi-Fainaro R, Hailu H, Davies JW, Summerford C, Duncan R. PDEPT: polymer-directed enzyme prodrug therapy. 2. HPMA copolymer-β-lactamase and HPMA copolymer-C-Dox as a model combination. *Bioconjug Chem* 2003, 14:797–804.
83. Vasey PA, Kaye SB, Morrison R, Twelves C, Wilson P, Duncan R, Thomson AH, Murray LS, Hilditch TE, Murray T, et al. Phase I clinical and pharmacokinetic study of PK1 [N-(2-hydroxypropyl)methacrylamide copolymer doxorubicin]: first member of a new class of chemotherapeutic agents—drug-polymer conjugates. *Clin Cancer Res* 1999, 5:83–94.
84. Seymour LW, Ferry DR, Anderson D, Hesslewood S, Julyan PJ, Poyner R, Doran J, Young AM, Burtles S, Kerr DJ. Hepatic drug targeting: phase i evaluation of polymer-bound doxorubicin. *J Clin Oncol* 2002, 20:1668–1676.
85. Meerum Terwogt JM, ten Bokkel Huinink WW, Schellens JH, Schot M, Mandjes IA, Zurlo MG, Rocchetti M, Rosing H, Koopman FJ, Beijnen JH. Phase I clinical and pharmacokinetic study of PNU166945, a novel water-soluble polymer-conjugated prodrug of paclitaxel. *Anti-Cancer Drugs* 2001, 12:315–323.
86. Singer JW. Paclitaxel poliglumex (XYOTAX™, CT-2103): a macromolecular taxane. *J Controlled Release* 2005, 109:120–126.
87. Bhatt R, de Vries P, Tulinsky J, Bellamy G, Baker B, Singer JW, Klein P. Synthesis and in vivo antitumor activity of poly(L-glutamic acid) conjugates of 20(*S*)-camptothecin. *J Med Chem* 2003, 46:190–193.
88. Danhauser-Riedl S, Hausmann E, Schick H-D, Bender R, Dietzfelbinger H, Rastetter J, Hanauske AR. Phase I clinical and pharmacokinetic trial of dextran conjugated doxorubicin (AD-70, DOX-OXD). *Invest New Drugs* 1993, 11:187–195.
89. Lee KS, Chung HC, Im SA, Park YH, Kim CS, Kim S-B, Rha SY, Lee MY, Ro J. Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast cancer. *Breast Cancer Res Treat* 2008, 108:241–250.
90. Lim WT, Tan EH, Toh CK, Hee SW, Leong SS, Ang PCS, Wong NS, Chowbay B. Phase I pharmacokinetic study of a weekly liposomal paclitaxel formulation (Genexol®-PM) in patients with solid tumors. *Ann Oncol* 2010, 21:382–388.
91. Hawkins MJ, Soon-Shiong P, Desai N. Protein nanoparticles as drug carriers in clinical medicine. *Adv Drug Deliv Rev* 2008, 60:876–885.
92. Hrkach J, Hoff DV, Ali MM, Andrianova E, Auer J, Campbell T, De Witt D, Figa M, Figueiredo M, Horhota A, et al. Preclinical development and clinical translation of a PSMA-targeted Docetaxel nanoparticle with a differentiated pharmacological profile. *Sci Transl Med* 2012, 4:128ra39.
93. Venne A, Li S, Mandeville R, Kabanov A, Alakhov V. Hypersensitizing effect of Pluronic L61 on cytotoxic activity, transport, and subcellular distribution of doxorubicin in multiple drug-resistant cells. *Cancer Res* 1996, 56:3626–3629.
94. Batrakova EV, Li S, Elmquist WF, Miller DW, Alakhov VY, Kabanov AV. Mechanism of sensitization of MDR cancer cells by Pluronic block copolymers: selective energy depletion. *Br J Cancer* 2001, 85:1987–1997.
95. Zhang Y, Jeon M, Rich LJ, Hong H, Geng J, Zhang Y, Shi S, Barnhart TE, Alexandridis P, Huizinga JD, et al. Non-invasive multimodal functional imaging of the intestine with frozen micellar naphthalocyanines. *Nat Nanotechnol* 2014, 9:631–638.

96. Nanjwade BK, Bechra HM, Derkar GK, Manvi FV, Nanjwade VK. Dendrimers: emerging polymers for drug-delivery systems. *Eur J Pharm Sci* 2009, 38:185–196.
97. Lee CC, MacKay JA, Fréchet JMJ, Szoka FC. Designing dendrimers for biological applications. *Nat Biotechnol* 2005, 23:1517–1526.
98. Rupp R, Rosenthal SL, Stanberry LR. VivaGel™ (SPL7013 Gel): a candidate dendrimer—microbicide for the prevention of HIV and HSV infection. *Int J Nanomedicine* 2007, 2:561–566.
99. Mcgowan I, Gomez K, Bruder K, Febo I, Chen BA, Richardson BA, et al. Phase 1 randomized trial of the vaginal safety and acceptability of SPL7013 gel (VivaGel®) in sexually active young women (MTN-004). *AIDS Lond Engl* 2011, 25:1057–1064.
100. Auffan M, Rose J, Bottero J-Y, Lowry GV, Jolivet J-P, Wiesner MR. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. *Nat Nanotechnol* 2009, 4:634–641.
101. Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA. Gold nanoparticles for biology and medicine. *Angew Chem Int Ed* 2010, 49:3280–3294.
102. Wu G, Mikhailovsky A, Khant HA, Fu C, Chiu W, Zasadzinski JA. Remotely triggered liposome release by near-infrared light absorption via hollow gold nanoshells. *J Am Chem Soc* 2008, 130:8175–8177.
103. Huang X, El-Sayed IH, Qian W, El-Sayed MA. Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods. *J Am Chem Soc* 2006, 128:2115–2120.
104. Murphy CJ, Gole AM, Stone JW, Sisco PN, Alkilany AM, Goldsmith EC, Baxter SC. Gold nanoparticles in biology: beyond toxicity to cellular imaging. *Acc Chem Res* 2008, 41:1721–1730.
105. Paciotti GF, Myer L, Weinreich D, Goia D, Pavel N, McLaughlin RE, Tamarkin L. Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery. *Drug Deliv* 2004, 11:169–183.
106. Libutti SK, Paciotti GF, Byrnes AA, Alexander HR, Gannon WE, Walker M, Seidel GD, Yuldasheva N, Tamarkin L. Phase I and pharmacokinetic studies of CYT-6091, a novel PEGylated colloidal gold-rhTNF nanomedicine. *Clin Cancer Res* 2010, 16:6139–6149.
107. Ito A, Shinkai M, Honda H, Kobayashi T. Medical application of functionalized magnetic nanoparticles. *J Biosci Bioeng* 2005, 100:1–11.
108. Cai W, Shin D-W, Chen K, Gheysens O, Cao Q, Wang SX, Gambhir SS, Chen X. Peptide-labeled near-infrared quantum dots for imaging tumor vasculature in living subjects. *Nano Lett* 2006, 6:669–676.
109. Byers RJ, Hitchman ER. Quantum dots brighten biological imaging. *Prog Histochem Cytochem* 2011, 45:201–237.
110. Resch-Genger U, Grabolle M, Cavaliere-Jaricot S, Nitschke R, Nann T. Quantum dots versus organic dyes as fluorescent labels. *Nat Methods* 2008, 5:763–775.
111. Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP, Ge N, Frank Peale F, Bruchez MP. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. *Nat Biotechnol* 2003, 21:41–46.
112. Park KH, Chhowalla M, Iqbal Z, Sesti F. Single-walled carbon nanotubes are a new class of ion channel blockers. *J Biol Chem* 2003, 278:50212–50216.
113. Klumpp C, Kostarelos K, Prato M, Bianco A. Functionalized carbon nanotubes as emerging nanovectors for the delivery of therapeutics. *Biochim Biophys Acta* 2006, 1758:404–412.
114. Wang J. Carbon-nanotube based electrochemical biosensors: a review. *Electroanalysis* 2005, 17:7–14.
115. Gao W, Chan JM, Farokhzad OC. pH-responsive nanoparticles for drug delivery. *Mol Pharm* 2010, 7:1913–1920.
116. Delie F, Blanco-Príeto MJ. Polymeric particulates to improve oral bioavailability of peptide drugs. *Molecules* 2005, 10:65–80.
117. Damgé C, Maincent P, Ubrich N. Oral delivery of insulin associated to polymeric nanoparticles in diabetic rats. *J Controlled Release* 2007, 117:163–170.
118. Chen M-C, Sonaje K, Chen K-J, Sung H-W. A review of the prospects for polymeric nanoparticle platforms in oral insulin delivery. *Biomaterials* 2011, 32:9826–9838.
119. Hsu PP, Sabatini DM. Cancer cell metabolism: Warburg and beyond. *Cell* 2008, 134:703–707.
120. Kratz F, Müller IA, Ryppa C, Warnecke A. Prodrug strategies in anticancer chemotherapy. *ChemMedChem* 2008, 3:20–53.
121. Lee ES, Bae YH. Recent progress in tumor pH targeting nanotechnology. *J Controlled Release* 2008, 132:164–170.
122. Meng F, Zhong Y, Cheng R, Deng C, Zhong Z. pH-sensitive polymeric nanoparticles for tumor-targeting doxorubicin delivery: concept and recent advances. *Nanomedicine* 2014, 9:487–499.
123. Chu C-J, Szoka FC. pH-sensitive liposomes. *J Liposome Res* 1994, 4:361–395.
124. Sethuraman VA, Bae YH. TAT peptide-based micelle system for potential active targeting of anti-cancer agents to acidic solid tumors. *J Controlled Release* 2007, 118:216–224.
125. Lee ES, Gao Z, Kim D, Park K, Kwon IC, Bae YH. Super pH-sensitive multifunctional polymeric micelle for tumor pHe specific TAT exposure and multidrug resistance. *J Controlled Release* 2008, 129:228–236.

126. Lovell JF, Zheng G. Activatable smart probes for molecular optical imaging and therapy. *J Innov Opt Health Sci* 2008, 1:45–61.

127. Lovell JF, Liu TWB, Chen J, Zheng G. Activatable photosensitizers for imaging and therapy. *Chem Rev* 2010, 110:2839–2857.

128. De la Rica R, Aili D, Stevens MM. Enzyme-responsive nanoparticles for drug release and diagnostics. *Adv Drug Deliv Rev* 2012, 64:967–978.

129. Delcea M, Möhwald H, Skirtach AG. Stimuli-responsive LbL capsules and nanoshells for drug delivery. *Adv Drug Deliv Rev* 2011, 63:730–747.

130. Andresen TL, Jensen SS, Kaasgaard T, Jorgensen K. Triggered activation and release of liposomal prodrugs and drugs in cancer tissue by secretory phospholipase A2. *Curr Drug Deliv* 2005, 2:353–362.

131. Lovell JF, Jin CS, Huynh E, MacDonald TD, Cao W, Zheng G. Enzymatic regioselection for the synthesis and biodegradation of porphysome nanovesicles. *Angew Chem* 2012, 124:2479–2483.

132. Zhou H, Yan H, Senpan A, Wickline SA, Pan D, Lanza GM, Pham CTN. Suppression of inflammation in a mouse model of rheumatoid arthritis using targeted lipase-labile fumagillin prodrug nanoparticles. *Biomaterials* 2012, 33:8632–8640.

133. Pan D, Schmieder AH, Wang K, Yang X, Senpan A, Cui G, Killgore K, Kim B, Allen JS, Zhang H, et al. Anti-angiogenesis therapy in the Vx2 rabbit cancer model with a lipase-cleavable Sn 2 taxane phospholipid prodrug using $\alpha(v)\beta^3$-targeted theranostic nanoparticles. *Theranostics* 2014, 4:565–578.

134. Wong C, Stylianopoulos T, Cui J, Martin J, Chauhan VP, Jiang W, Popović Z, Jain RK, Bawendi MG, Fukumurab D. Multistage nanoparticle delivery system for deep penetration into tumor tissue. *Proc Natl Acad Sci U S A* 2011, 108:2426–2431.

135. Yavlovich A, Singh A, Tarasov S, Capala J, Blumenthal R, Puri A. Design of liposomes containing photopolymerizable phospholipids for triggered release of contents. *J Therm Anal Calorim* 2009, 98:97–104.

136. Bae YH, Okano T, Hsu R, Kim SW. Thermo-sensitive polymers as on-off switches for drug release. *Makromol Chem Rapid Commun* 1987, 8:481–485.

137. Yatvin MB, Weinstein JN, Dennis WH, Blumenthal R. Design of liposomes for enhanced local release of drugs by hyperthermia. *Science* 1978, 202:1290–1293.

138. Fomina N, Sankaranarayanan J, Almutairi A. Photochemical mechanisms of light-triggered release from nanocarriers. *Adv Drug Deliv Rev* 2012, 64:1005–1020.

139. Brazel CS. Magnetothermally-responsive nanomaterials: combining magnetic nanostructures and thermally-sensitive polymers for triggered drug release. *Pharm Res* 2009, 26:644–656.

140. Needham D, Anyarambhatla G, Kong G, Dewhirst MW. A new temperature-sensitive liposome for use with mild hyperthermia: characterization and testing in a human tumor xenograft model. *Cancer Res* 2000, 60:1197–1201.

141. Kong G, Anyarambhatla G, Petros WP, Braun RD, Colvin OM, Needham D, Dewhirst MW. Efficacy of liposomes and hyperthermia in a human tumor xenograft model: importance of triggered drug release. *Cancer Res* 2000, 60:6950–6957.

142. Miller AD. Lipid-based nanoparticles in cancer diagnosis and therapy. *J Drug Deliv* 2013, 2013:e165981.

143. Poon RT, Borys N. Lyso-thermosensitive liposomal doxorubicin: a novel approach to enhance efficacy of thermal ablation of liver cancer. *Expert Opin Pharmacother* 2009, 10:333–343.

144. Hong CW, Libutti SK, Wood BJ. Liposomal doxorubicin plus radiofrequency ablation for complete necrosis of a hepatocellular carcinoma. *Curr Oncol* 2013, 20:e274–e277.

145. Hu S-H, Chen S-Y, Liu D-M, Hsiao C-S. Core/single-crystal-shell nanospheres for controlled drug release via a magnetically triggered rupturing mechanism. *Adv Mater* 2008, 20:2690–2695.

146. Babincová M, Cicmanec P, Altanerová V, Altaner C, Babinec P. AC-magnetic field controlled drug release from magnetoliposomes: design of a method for site-specific chemotherapy. *Bioelectrochemistry* 2002, 55:17–19.

147. Amstad E, Kohlbrecher J, Müller E, Schweizer T, Textor M, Reimhult E. Triggered release from liposomes through magnetic actuation of iron oxide nanoparticle containing membranes. *Nano Lett* 2011, 11:1664–1670.

148. Derfus AM, von Maltzahn G, Harris TJ, Duza T, Vecchio KS, Ruoslahti E, Bhatia SN. Remotely triggered release from magnetic nanoparticles. *Adv Mater* 2007, 19:3932–3936.

149. Bringas E, Köysüren Ö, Quach DV, Mahmoudi M, Aznar E, Roehling JD, Marcos MD, Martínez-Máñez R, Stroeve P. Triggered release in lipid bilayer-capped mesoporous silica nanoparticles containing SPION using an alternating magnetic field. *Chem Commun* 2012, 48:5647–5649.

150. Kobayashi T. Cancer hyperthermia using magnetic nanoparticles. *Biotechnol J* 2011, 6:1342–1347.

151. Kopechek JA, Abruzzo TM, Wang B, Chrzanowski SM, Smith DAB, Kee PH, Huang S, Collier JH, McPherson DD, Holland CK. Ultrasound-mediated release of hydrophilic and lipophilic agents from echogenic liposomes. *J Ultrasound Med* 2008, 27:1597–1606.

152. De Geest BG, Skirtach AG, Mamedov AA, Antipov AA, Kotov NA, De Smedt SC, Sukhorukov GB. Ultrasound-triggered release from multilayered capsules. *Small* 2007, 3:804–808.

153. Kim H-J, Matsuda H, Zhou H, Honma I. Ultrasound-triggered smart drug release from a

poly(dimethylsiloxane)–mesoporous silica composite. *Adv Mater* 2006, 18:3083–3088.

154. Rapoport NY, Christensen DA, Fain HD, Barrows L, Gao Z. Ultrasound-triggered drug targeting of tumors in vitro and in vivo. *Ultrasonics* 2004, 42:943–950.
155. Dromi S, Frenkel V, Luk A, Traughber B, Angstadt M, Bur M, Poff J, Xie J, Libutti SK, Li KC, et al. Pulsed-high intensity focused ultrasound and low temperature–sensitive liposomes for enhanced targeted drug delivery and antitumor effect. *Clin Cancer Res* 2007, 13:2722–2727.
156. Schroeder A, Honen R, Turjeman K, Gabizon A, Kost J, Barenholz Y. Ultrasound triggered release of cisplatin from liposomes in murine tumors. *J Controlled Release* 2009, 137:63–68.
157. Tong R, Kohane DS. Shedding light on nanomedicine. *WIREs Nanomed Nanobiotechnol* 2012, 4:638–662.
158. Koçer A, Walko M, Meijberg W, Feringa BL. A light-actuated nanovalve derived from a channel protein. *Science* 2005, 309:755–758.
159. Leung SJ, Romanowski M. Light-activated content release from liposomes. *Theranostics* 2012, 2:1020–1036.
160. Yavlovich A, Smith B, Gupta K, Blumenthal R, Puri A. Light-sensitive lipid-based nanoparticles for drug delivery: design principles and future considerations for biological applications. *Mol Membr Biol* 2010, 27:364–381.

