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Nanoscale Drug Delivery Systems for Enhanced Drug Penetration into Solid Tumors: Current Progress and Opportunities

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Abstract

Poor penetration of anticancer drugs into solid tumors significantly limits their efficacy. This phenomenon has long been observed for small-molecule chemotherapeutics, and it can be even more pronounced for nanoscale therapies. Nanoparticles have enormous potential for the treatment of cancer due to their wide applicability as drug delivery and imaging vehicles and their size-dependent accumulation into solid tumors by the enhanced permeability and retention (EPR) effect. Further, synthetic nanoparticles can be engineered to overcome barriers to drug delivery. Despite their promise for the treatment of cancer, relatively little work has been done to study and improve their ability to diffuse into solid tumors following passive accumulation in the tumor vasculature. In this review, we present the complex issues governing efficient penetration of nanoscale therapies into solid tumors. The current methods available to researchers to study nanoparticle penetration into malignant tumors are described, and the most recent works studying the penetration of nanoscale materials into solid tumors are summarized. We conclude with an overview of the important nanoparticle design parameters governing their tumor penetration, as well as by highlighting critical directions in this field.

Keywords

Tumor penetration; solid tumors; nanoparticles; cancer; liposomes; polymeric micelles; drug delivery

I. INTRODUCTION

Limited penetration and poor spatial distribution of drugs throughout solid tumors represent significant barriers to their anticancer efficacy. Several conventional small-molecule chemotherapeutics, including doxorubicin,[1,2] paclitaxel,[3,4] and other clinically relevant compounds,[5] are known to exhibit poor distribution throughout solid tumors. These drugs remain localized to regions immediately surrounding blood vessels, leaving large regions of the tumor untouched by the therapy. Their poor tumor distribution may significantly impair their efficacy, contributing to disease recurrence and the administration of high drug doses that cause adverse effects in cancer patients. Improving the distribution of drugs in solid tumors is thought to improve their therapeutic index for the treatment of human disease.[6]

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With the increasing application of nanoscale materials for cancer drug delivery and imaging purposes, the importance of tumor penetration by drugs becomes more pronounced. As nanoscale materials are orders of magnitude larger than conventional chemotherapeutic compounds, their transport and diffusion through tumor tissue is even more limited. On the other hand, nano-medicines can be engineered with functionalities to mediate more effective transport within tumors. Whereas significant progress has been made to understand and improve the tumor transport of small-molecule and antibody therapeutics, [6,7] much less work has been done to understand similar phenomena for nanoscale materials.[8,9]

This review focuses on the current state of nanoscale drug delivery systems for enhanced drug distribution throughout solid tumors. The properties of solid tumors that hinder homogeneous drug delivery are discussed first. Then the available experimental and theoretical methods to study drug distribution in solid tumors are reviewed, with an emphasis on applications to nanoscale drug delivery systems. Finally, the current literature describing methods employed by researchers to understand and overcome the poor tumor transport of nanoscale materials including liposomes, inorganic nanoparticles, and synthetic polymeric systems is reviewed, highlighting the design parameters that are important for each unique type of delivery system. In this review, we define tumor penetration as the transport process that occurs after a nanoparticle drug has left the tumor vasculature (by extravasation) and has entered the adjacent tumor tissue. We discuss methods to study and improve nanoparticle transport through the tumor tissue (both extracellular matrix and tumor cells) after the drug has reached the surface of the tumor.

II. TUMOR PROPERTIES HINDERING NANOSCALE DRUG TRANSPORT

Compared with healthy tissues, solid tumors have unique structural properties that restrict transport and distribution of drug compounds throughout malignant tissue. Several reviews have thoroughly discussed the architectural features of solid tumors that hinder drug transport;[6–9] thus, only a brief overview of these features is discussed here.

A. Abnormal Vasculature

One critical feature of tumors that enables them to have an abnormal survival advantage is their ability to sustain angiogenesis, or to acquire their own blood supply.[10] For cells to survive, they should be within 100 μm of a blood vessel, allowing transport of oxygen and critical nutrients by molecular diffusion. In the development of healthy tissues, the formation of blood vessels is carefully regulated to ensure that there is an ample blood supply for all cells. Malignant tumors, however, are formed abnormally in the midst of healthy tissues, and therefore they must acquire their own blood supply via angiogenesis to progress to a large size.[10] As the acquisition of a blood supply is abnormal in tumor tissue, the structure of the tumor vasculature is poorly organized compared with healthy tissues (Figure 1). The blood vessels in solid tumors are more heterogeneous in distribution, size, and are more permeable than in healthy tissue.[7] A consequence of heterogeneous vascularization is that some regions of the tissue are less accessible than others to oxygen, nutrients, and therapeutic compounds. One of the theories behind the use of antiangiogenic drugs is to “normalize” the tumor vasculature, rendering it more permissive to accompanying pharmacologic treatment.[9]

The abnormal tumor vasculature has commonly been thought to confer an advantage for the accumulation of nanoscale therapeutics due to the well-known enhanced permeability and retention (EPR) effect. Tumor vasculature has excessive leakage compared with healthy vasculature, which is a result of large pores in the blood vessels. Nanoscale therapeutics (on the order of 100 nm) tend to accumulate in the leaky pores of tumors more extensively than their small-molecule counterparts due to blood circulation. This is commonly described as

"passive tumor-targeting," an effect that is amplified by the increased retention that is a consequence of poor lymphatic drainage of tumors.[11] It is important to note that, while the EPR effect may guide nanoparticle therapeutics to the tumor vasculature more rapidly than small molecules, the vast majority of intravenously administered drugs (both nanoparticle and small- molecule) accumulate in other organs including the liver, spleen, and lungs. A thorough discussion of the EPR effect in drug delivery to tumors has recently been provided. [12] Here, we focus our review on the mechanisms of nanoparticle tumor transport after the particles have reached the tumor site following systemic or local administration. Although the abnormal tumor vas may be advantageous for the tumor accumulation of nanoscale therapeutics, the ability of these materials to transport across vascular walls (extravasate) and to subsequently diffuse into the surrounding tumor tissue poses a significant challenge for these emerging therapeutics. Extravasation of nanoparticle drug carriers from tumor blood vessels is the first significant barrier to their tumoral delivery, a process that has been thoroughly reviewed elsewhere.[13]

B. Elevated Interstitial Fluid Pressure (IFP)

Another significant barrier to drug transport from blood vessels is the elevated interstitial fluid pressure (IFP) found in solid tumors. In healthy tissues, the IFP is carefully regulated so that the total (hydrostatic plus osmotic) pressure gradient between the blood vessels and tissue promotes fluid flow and nutrient transport out of blood vessels and into cells. However, in the case of some solid tumors, there is a slightly elevated IFP resulting from abnormal blood vessel and extracellular architecture, along with high cell density and insufficient lymphatic drainage.[14] Inefficient uptake of therapeutic agents is a consequence of this high tumoral IFP; an unfavorable pressure gradient exists between the tissue and blood vessels, which may hinder fluid flow into the tumor from blood capillaries. Several agents, including antagonists of vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and transforming growth factor beta (TGFβ), have been used to reduce the IFP within tumors; doing so has improved the tumoral transport and penetration of macromolecular proteins[15] and nanomedicines.[14]

C. Dense Extracellular Matrix (ECM)

Following extravasation from blood vessels, the transport of particles through the extracellular space to reach malignant cells poses the next barrier to drug delivery. The abnormal tumor vasculature results in a generally low blood supply within tumors; there is insufficient convective transport of drugs within tumors, forcing drugs to be transported primarily by diffusion.[16] However, diffusion through the tumor space is hindered by the complex structure of the extracellular matrix (ECM) in solid tumors. The extracellular space in solid tumors is comprised of fibrous macromolecules, including collagen and glycosaminoglycans (GAGs). In particular, the collagen content in solid tumors is significantly higher than in healthy tissues, resulting in a relatively dense tumoral extracellular space.[8] Small-molecule drugs are able to diffuse through this protein matrix due to their small size; however, the large size of nanoscale therapeutics severely impairs their mobility, causing them to localize in regions immediately surrounding blood vessels.[9] Several researchers have successfully improved the transport of nanoscale therapeutics through the dense ECM by incorporating agents that can degrade or normalize ECM proteins.[17–24] Some specific examples using this approach are discussed in later sections.

III. TECHNIQUES FOR STUDYING DRUG DISTRIBUTION WITHIN SOLID TUMORS

Several experimental and theoretical approaches have been developed to study the distribution of drugs (both small-molecule and macromolecular) throughout solid tumors.

These methods have enabled researchers to visualize the distribution of drugs throughout tumors both *in vitro* and *in vivo*. Further, the use of mathematical models has enabled the quantification of physical transport parameters such as effective diffusion coefficients, providing a quantitative understanding of how drug design parameters impact their tumor transport. The available techniques for studying drug distribution are reviewed in the following sections, with a particular emphasis on how these methods have been applied to understanding the distribution of nanomedicines in solid tumors.

A. *In Vitro* Three-Dimensional (3D) Tumor Models

Three-dimensional tumor models have been used widely for studying anticancer medicines due to their ease of formation from commercially available cancer cell lines and their ability to provide a more realistic model of the *in vivo* tumor microenvironment than cells cultured on conventional two-dimensional plates. Various types of 3D culture models exist to study tumors including multicellular layers, multicellular spheroids, and collagen scaffold-based cultures, all of which have been reviewed thoroughly elsewhere.[25–28] Although 3D cell culture models have been used extensively to study many aspects of solid tumors, they have proven particularly useful in understanding the tumoral transport of anticancer nanomedicines.[29,30]

Multicellular layers (MCLs) have been used to study the tumor penetration and transport of small-molecule drugs. Their simple geometry makes them amenable to use in a diffusion chamber apparatus, enabling quantitative determination of drug diffusion coefficients across multiple layers of tumor cells. Specifically, MCL diffusion models have been used to study the diffusive transport rates of the small-molecule anticancer drugs tirapazamine,[31] vinblastine,[32] a variety of anthracycline analogues including doxorubicin,[33] and several other commonly used chemotherapeutic agents including paclitaxel and methotrexate.[34] The diffusion of doxorubicin through MCLs has also been described using a mathematical transport model, which may be a useful tool for predicting drug penetration into tumors or for extracting quantitative parameters from experimental studies.[35] The kinetic information obtained for tirapazamine analogues using MCLs (including MCL diffusion rates and rates of cellular metabolism) was found to be predictive of *in vivo* drug efficacy in a mouse xenograft model.[31] Although MCLs have not been used specifically for nanoscale materials, they could easily be used to study these materials. An imaging technique using confocal microscopy to track nanoparticles through 3D tissue engineered cell culture models has been recently described[36] and may be applicable to the study of nanoparticle distribution through MCLs. This relatively simple cell culture model coupled with effective imaging and mathematical modeling techniques may serve as a useful method to screen potential nanoparticle drug candidates for their antitumor efficacy prior to performing *in vivo* studies.

Multicellular spheroids are probably the most widely used 3D cell culture model for studying the penetration and transport of anticancer nanomedicines. There are several standard methods for generating spheroids from tumor cells: the hanging drop method,[37] culture on a non-adhesive substrate to promote the formation of multicellular bodies, [38,39] and spinner flask culture.[40] Some methodological improvements have been proposed to improve the growth and formation of spheroids. These include the incorporation of a transient polycation linker during the spheroid formation phase to generate more consistent spheroids for drug screening applications,[41] or using a hydrogel micromold to control their shape and size.[42] Spheroids have been used extensively to study the penetration of nanoscale liposomes,[43–46] gene and siRNA delivery vehicles,[47–52] and other nanoparticles for drug delivery and imaging applications.[19,53–57] Further, their spherical geometry has made them amenable for developing descriptive mathematical models describing drug transport of macromolecular[58–56] and nanoscale therapeutics.[29,61] Spheroids have already proven to be a

critical tool in studying the penetration of nanoscale materials and will likely continue as a valuable method to screen and understand the tumor transport of nanoparticle drugs.

Gels comprised of ECM proteins including collagen, laminin, or Matrigel (a basement membrane extract comprising several tumor ECM proteins) have been used to replicate the architecture of solid tumors. Gel-embedded cell cultures have been used to study cancer morphogenesis and drug resistance in three dimensions[27] and have more recently been applied to study drug penetration into solid tumors. Gels can be used as a scaffold to support the growth of 3D cell cultures, or they can be used without cells to assess drug penetration in the tumor ECM. One study found that collagen gels alone (without the use of any cells) could provide similar resistance to macromolecule penetration as observed in solid tumors.[62] Matrigel has also been used in an *in vitro* setting to represent the tumor ECM in conjunction with Caco-2 cells, and it was found to be a reasonable surrogate for the tumor penetration of small-molecule thioxanthones.[63] Because gels are simple *in vitro* models that provide reasonable similarity to solid tumors, they can serve as useful experimental tools for screening the diffusive characteristics of nanoscale drug compounds.

B. *In Vivo* Quantitative Imaging

Although *in vitro* models can be informative for preliminary studies, it is critical to develop approaches to study tumor drug distribution *in vivo*, as the *in vivo* tumor microenvironment cannot be perfectly replicated using any *in vitro* cell culture model. Several imaging techniques have been applied to study nanoparticle distribution *in vivo,* including magnetic resonance imaging (MRI), X-ray computed tomography (CT), and various forms of fluorescent or multiphoton confocal microscopy. Each of these techniques has its advantages and limitations, and a technique should be carefully selected for *in vivo* studies depending on the desired resolution, ability to acquire quantitative data, and whether or not an invasive technique is plausible for a particular study.

1. In vivo MRI and CT Scans—Non-invasive imaging and diagnostic techniques such as MRI and CT scanning are widely used in a clinical setting for the diagnosis and observation of many human diseases, and they have recently found application in the study of the intratumoral distribution of nanoscale drug carriers. When such non-invasive imaging techniques are used to characterize drug delivery, the approach may be rapidly translatable to clinical practice. The capability to simultaneously administer diagnostic imaging with therapeutic platforms has brought about a new field known as "theranostics."[64,65]

MRI has been used to quantify the concentration of doxorubicin throughout solid tumors *in vivo* after its liposomal delivery.[66–68] Liposomes were co-loaded with both doxorubicin and manganese, an MRI contrast agent, and the concentration of doxorubicin within the solid tumors was calculated from the measured manganese concentration. The limitation of this technique is that colocalization of doxorubicin and the MRI contrast agent is assumed, when in reality the pharmacokinetics and systemic clearances of contrast agents and drugs are highly variable.[66] However, this approach has the advantage of being non-invasive and can provide reasonable spatial and quantitative information regarding the tumoral delivery of nanoscale materials *in vivo*. More recently, a quantitative technique using MRI was developed whereby the tumoral distribution and drug release from poly($_{D,L}$-lactide-*co*-glycolide) (PLGA) nanoparticles was achieved.[69] Positive and negative contrast agents were both incorporated into the nanoparticles to distinguish between intact nanocarriers and released drug. A small unilamellar vesicle (SUV) system was also developed to facilitate multimodal imaging of tumors by incorporating an MRI contrast agent with a near-IR dye for optical imaging.[70] The approach of incorporating an MRI contrast agent into a drug delivery system may be useful for improving the capabilities of MRI to evaluate the tumoral

distribution of engineered therapeutic agents. It may also be useful to employ nanoscale particles, such as iron oxide nanoparticles, which are inherently detectable by MRI, as drug delivery systems to enable simultaneous delivery and imaging capabilities within a single-particle entity.

A single-photon emission computed tomography (microSPECT/CT) scan has been applied to track the tumoral distribution of nanoscale polymeric micelles *in vivo*.[71] The polymeric micelles were covalently labeled with the radionucleotide indium-111 to facilitate CT imaging, and mice were imaged using non-invasive microSPECT/CT to show inhomogeneous distribution of polymeric micelles within the solid tumor tissue. This non-invasive approach could be extended to study the delivery of drug cargo by other nanoscale materials.

2. Fluorescence Microscopy Imaging—Fluorescence techniques are particularly attractive for studying the distribution of nanoscale drugs in solid tumors due to the potential for simultaneous visualization of several fluorescent dyes with good spatial resolution. However, imaging tumors directly *in situ* with fluorescence microscopy techniques is challenging due to the shallow tissue penetration ability of most optical techniques, which is limited to ~200 μm.[72] In many cases, it is necessary to perform tumor excision prior to fluorescent microscopy.

Several studies have acquired informative data about the extravasation, distribution, and diffusion of nanoscale drugs within solid tumors by the analysis of excised tumors with fluorescent microscopy. For instance, multiplexed fluorescence imaging was used to show the co-localization of polymeric nanospheres with the tumor vasculature from tumors excised from a mouse model of Lewis lung carcinoma.[73] Multimodal imaging has provided rich information regarding intratumoral distribution, using a polymeric nanoparticle system for the co-delivery of quantam dots and iron oxides. In this case, MRI was used to visualize drug localization *in situ*, and improved intratumoral resolution was achieved with confocal microscopy following the excision of tumors from mice.[74] A multiphoton imaging technique was used on excised human tumors to quantitatively measure the diffusion coefficients of macromolecular proteins,[75] showing the applicability of this technique to measurements in human tissue. Although these invasive *ex vivo* studies do provide detailed information about the distribution of nanoscale materials in solid tumors, it is necessary to develop techniques that will enable the imaging of tumors *in situ*.

Some fluorescent microscopy techniques have enabled the visualization of drugs in solid tumors *in vivo* without the need to excise the tumor. The potential for *in vivo* tumor imaging methods was identified more than a decade ago when liposome extravasation from tumor vascular networks was visualized using fluorescence video microscopy.[76] Recently, more advanced microscopy techniques such as multiphoton laser scanning microscopy have enabled the detailed resolution of tumor architecture *in vivo* up to ~300 μm in depth.[77] Multiphoton methods have also been used to extract intratumoral transport parameters including diffusion coefficients and concentration profiles of nanoscale liposomes *in vivo*,[78] and they have been used to spatially discern the tumor vascular localization of nanoscale particles.[79] Intravital microscopy (IVM) has historically been used to study physiological processes in live animals and has been applied more recently to imaging the distribution of anticancer drugs in solid tumors *in situ*.[80] IVM has been used to track the tumor binding and transport of nanoscale quantam dots *in vivo*,[81,82] and it has also been applied to visualizing the tumoral drug delivery by carbon nanotubes.[83] Another recently developed method called microfiberoptic epifluorescence photobleaching (MFEP) enables the quantification of drug diffusion deep within tumors (depths greater than 1 mm), but involves the insertion of a fiber optic tip into the tumor mass prior to imaging, which may disturb the drug distribution

in the tumor.[17,72] Nonetheless, this method has high resolution, is quantitative, and may provide useful information for readily accessible human tumors. Fluorescence microscopy techniques have also been used to measure the convective transport of nanoparticles through blood vessels in real time.[84] The ability to measure both convective transport, through monitoring blood flow, and diffusive transport, via drug distribution throughout solid tumors, arms researchers with the tools necessary to gain a thorough understanding of nanoparticle drug transport from injection to final tumor distribution.

C. Mathematical Modeling Approaches

In conjunction with experimental techniques, mathematical modeling approaches have been utilized to predict drug penetration in solid tumors. Models describing drug transport on the cell and tissue level, as well as those extending transport to the whole-body scale, have provided important insights into the properties and mechanisms governing drug transport in tumors.

A significant body of work modeling antibody penetration into solid tumors has yielded important design parameters for anticancer antibody therapeutics. Mathematical modeling approaches first identified a “binding-site barrier” to antibody transport in solid tumors, wherein antibodies displaying a high binding affinity to tumor cells exhibit reduced antibody penetration.[85] In this hypothesis, high-affinity molecules bind rapidly to tumor cells, leaving very few unbound molecules free to transport deep within solid tumors. Experimental support for this theory is provided by the finding that antibodies with a lower binding affinity to tumor cells exhibit more homogeneous penetration into guinea pig micrometastases[86] and colon cancer tumor spheroids[60] than antibodies with higher binding affinities. Models have also been developed describing the importance of other parameters such as antibody dosage,[59] antigen turnover rate,[58] and plasma clearance rate[87] on antibody penetration and retention into tumors. The knowledge gained from these models has enabled the prediction of antibody penetration based on the complex interplay between antibody molecular weight, size, and affinity.[88] These modeling approaches may be very useful in predicting the antitumor activity of anticancer drugs prior to performing any extensive experiments. The knowledge gained from the modeling of antibody distribution has been applied to studying the tumor distribution of small-molecule drugs[35,89] and nanoparticles.[29,90] Large-scale pharmacokinetic modeling has also been employed to describe the whole-body distribution of macromolecular agents.[91]

IV. PARAMETERS GOVERNING EFFICIENT TUMOR PENETRATION: SMALL-MOLECULE DRUGS AND ANTIBODIES

The transport properties governing tumor penetration of antibodies and small-molecule therapeutics provide important guidelines that can be extended for the design of nanoscale therapeutics. The process of tumor penetration begins with convective transport through blood vessels, extravasation through vessel walls, and finally diffusive transport through the tumor mass. The primary mode of drug transport in the tumor mass for both small-molecule and macromolecular drugs is diffusion because there remains no opportunity for convective transport once drugs have left the vasculature.

The properties governing diffusive tumor transport include molecular (free) diffusivity, hindrance by the tumor interstitium, a cell-membrane barrier resulting from high cell density, cell binding affinity, cell internalization and metabolism kinetics, and systemic clearance.[92,93] Diffusion within a tumor is also inhibited by the binding of molecules to the cell surface and by cellular internalization of molecules (Figure 2). However, it is imperative that chemotherapeutics have some affinity to tumor cells so that they may enter and exert

their pharmacologic effects. Thus, it is important to design drugs or delivery systems such that they strike a balance between efficient diffusion and cellular affinity, ensuring that the therapeutic will transport deep within the tumor, and will accumulate in quantities sufficient to elicit a therapeutic effect. The relative contributions of drug diffusion, cellular binding and internalization, and tumor clearance are known to govern the tumor penetration efficiency of antibody therapeutics.[92,93] Scaling analyses have suggested that if the rate of diffusion with binding is greater than both the systemic clearance rate and cell internalization rates, there will be efficient antibody tumor transport.[92] Being mindful of these important scaling analyses will aid researchers in rationally designing antitumor therapeutics with favorable tumor penetration properties.

The impact of drug architecture on tumor penetration and retention has also been investigated. In addition to binding affinity, the tumor distribution of anticancer therapeutics is influenced by molecular size, surface charge, and shape.[8,9] The accumulation of therapeutic antibodies in HER2-overexpressing breast tumors, for example, was studied as a function of antibody size, affinity, and shielding by PEGylation.[94] The delicate interplay between size and affinity was critical for tumor accumulation, with size governing the molecular diffusivity inversely. Two design regimes were observed to elicit the highest tumor accumulation (small proteins with high affinity or large proteins with low affinity). For a class of small molecules (anthraquinones), the net molecular charge influenced drug distribution in tumor spheroids, presumably by altering their rate of cellular uptake.[95] These design guidelines, which were initially derived for small molecules and antibodies, have been extended to understanding and improving tumor penetration for nanoscale materials. The following section focuses on how the structure of nanoscale therapeutics influences their tumor penetration.

V. THE EFFECT OF PARTICLE ARCHITECTURE ON TUMOR DISTRIBUTION

Significant progress has been made in understanding and improving the tumor distribution of nanoscale materials. Nanoscale drug delivery systems have enormous potential to revolutionize cancer therapy due to their inherent size advantages and their ability to be tailored chemically. Several classes of nanomaterials have been used for anticancer therapy including liposomes, polymeric or protein nanoparticles, and inorganic nanoparticles. The ability of these systems to penetrate into solid tumors for various applications, including chemotherapeutic drug delivery, the delivery of genes or antisense molecules, and for imaging or diagnostic purposes, has been investigated. As each class of nanoscale material has its own unique properties, it is important to examine these delivery systems individually to understand the distinct design parameters that are important for each particle type.

A. Polystyrene Beads

Polystyrene (PS) beads have been used as a model system to study the factors governing tumor transport of nanoscale materials. PS beads of sizes ranging from 20 to 100 nm were used to study the single particle trajectories through breast cancer xenografts in mice.[96] This study provided evidence that while particle size did impact diffusion through the tumors, the position of nanoparticles within tumors was also important. Diffusion through some regions of the tumor intersititium was limited due to the presence of a dense protein matrix, compared with other tumor regions where particle transport was more permissive. PS beads were also used as a model system to evaluate the effect of pulsed ultrasound on particle transport through breast cancer spheroids.[53] Pulsed ultrasound was found to increase the penetration of the beads through tumor spheroids, with particle size and charge also impacting tumor penetration in this case. The effect of collagenase treatment of tumor spheroids on PS bead penetration was also assessed, where collagenase increased the penetration of beads with sizes less than 100 nm in this spheroid system. [19] Similarly, when

PS nanoparticles were coinfused with hyaluronidase, a component used to degrade extracellular hyaluronan, an improvement in nanoparticle tumor distribution was observed.[21] These studies demonstrate the utility of PS beads as a model system to study and optimize the transport of nanoscale particles through solid tumors.

B. Liposomes

Liposomes have been studied extensively for the delivery of anticancer agents, mostly for their ability to encapsulate drugs of varying hydrophobicity and to extend their circulation time. In doing so, they have been found to reduce the undesired toxicity of encapsulated chemotherapy drugs.[97] There is a liposomal formulation for the delivery of doxorubicin, Doxil, that is used clinically for the treatment of ovarian cancer and multiple myeloma. [98] Despite their promise for the treatment of human disease, liposomes, including the clinically relevant formulation Doxil, exhibit poor penetration into solid tumors, delivering their drug cargo only to cells on the tumor periphery.[45,99] The premature release of doxorubicin prior to deep liposomal tumor penetration could limit its effectiveness by causing localized tumor-surface cytotoxicity.

Extensive studies have been performed to evaluate the effect of liposome size and charge on tumor penetration efficiency. The size of liposomes is an important parameter governing their tumor distribution, with smaller liposomes generally distributing more homogeneously through tumors than larger ones. Small unilamellar vesicles (less than 100 nm) exhibited the most homogeneous distribution in tumor spheroids compared with large multilamellar vesicles with sizes up to 1000 nm.[43] *In vivo*, liposomes ranging in size from 100 to 200 nm mediated the greatest tumor accumulation of a fluorescent dye among formulations ranging from 63 to 388 nm in size, presumably due to the EPR effect that is prominent in that size range.[100] More recently, small PEGylated phospholipids were generated in the 10–20 nm size range, which improved the tumor penetration and antitumor efficacy of doxorubicin, likely in part due to their small size.[101] It is well established that smaller liposomes generally have better tumor distribution than larger ones.

The surface charge of liposomes is another important factor in their tumor distribution that requires careful examination. Cationic liposomes have been found to accumulate in the tumor vasculature due to electrostatic interactions with the angiogenic endothelial cells found in tumor blood vessels.[102,103] However, highly charged cationic liposomes do not diffuse well into the tumor mass due to electrostatic binding to tumor cells and ECM components.[43,44] whereas less cationic or neutral liposomes have exhibited more efficient penetration into tumor spheroids *in vitro*[43,44] and extravasation from blood vessels *in vivo*.[103] Overall, maintaining a moderate cationic charge on liposomes seems to be favorable to maintaining their ability to accumulate in the tumor vasculature. Cationic liposomes are also useful for gene delivery applications because they are able to encapsulate and deliver anionic nucleic acids.[104] Functionalizing the constituent lipids with poly(ethylene glycol) (PEG) has shielded their cationic groups to prevent unfavorable electrostatic interactions with tumor cells and the ECM. PEGylated cationic liposomes have accumulated in the tumor vasculature *in vivo*[105–108] and have exhibited homogeneous tumor distribution.[107] This strategy of using PEGylation to somewhat shield the positive charge of cationic liposomes may serve as a very useful construct for designing liposomes that efficiently target and penetrate into solid tumors.

The sequential administration of PEGylated cationic liposomes has been another successful approach to improve their penetration into solid tumors.[108] Deep tumor penetration of oxaliplatinloaded liposomes was observed in mice bearing lung carcinoma tumors after tumors were sequentially dosed three times with the liposomes. Presumably, this was by enlarging the intratumoral interstitial space with the previous drug administrations. The use

of such a sequential dosing strategy may further improve the tumor distribution and ultimately the efficacy of liposome-based anticancer therapeutics.

Hyperthermia, or a localized increase in tissue temperature, has been used to increase both the vascular extravasation and tumor penetration of liposomal therapeutics. The extravasation of liposomes of 100 nm in size was significantly improved when increasing the body temperature of mice from 37°C to 42°C.[109,110] The use of hyperthermia to improve the efficacy of liposomal Doxil (doxorubicin liposomal) has also been evaluated clinically, where it was demonstrated to be a safe, effective treatment regimen for the treatment of ovarian cancer.[111]

The efficacy of Doxil was also improved by the administration of losartan, an FDA-approved agent that decreased the production of tumoral collagen. [24] The presence of losartan improved both the distribution and efficacy of Doxil against several murine tumor models. This promising approach may be useful in conjunction with other nanoparticle therapeutics, and it may be easily translatable to the clinic because it utilizes an FDA-approved drug to modulate the tumor microenvironment.

C. Amphiphilic Polymer Micelles

Amphiphilic macromolecules, or polymeric micelles, have found wide applicability for drug delivery purposes.[112,113] These synthetic materials can be constructed by grafting hydrophobic materials to a hydrophilic component (often PEG). These amphiphilic copolymers self-assemble in aqueous media to form micelles in the nanoscale size range. Unlike liposomes, micelles have a hydrophobic core rather than an aqueous core within a bilayer, as is the case for liposomes. Polymeric micelles are attractive materials for drug delivery applications because their structure can be easily modified and because they can be tailored to deliver a variety of drug cargo including chemotherapeutics, nucleic acids, or imaging agents. While polymeric micelles have met with success in the literature for drug delivery applications, they have been shown to exhibit poor penetration into and distribution throughout solid tumors.[114]

PEGylation is a widely used strategy for the formation of polymeric micelles because its hydrophilic nature renders polymeric micelles amphiphilic and because PEG makes the materials more biocompatible. In some cases, PEG has improved the ability of polymeric micelles to penetrate into solid tumors. When incorporated into a polymeric micelle, PEG tends to “shield” charged nanoparticles or drug cargo. This shielding effect is advantageous because it prevents the non-specific interactions of charged particles or drug cargo with tumor cells, enabling the micelles to penetrate deeper into tumors. For example, the enhanced penetration of doxorubicin by PEGylated block copolymer micelles has been demonstrated both in 3D cell culture models and *in vivo*, which may be attributed to this shielding effect.[54,55] PEG shielding of hyaluronic acid (HA) polymers also improved their ability to extravasate and distribute through solid tumors *in vivo*, improving their tumor accumulation by 1.6-fold.[115]

This shielding effect has proven particularly useful in delivering nucleic acids, which are large, anionic molecules that might have unwanted electrostatic interactions with tumor cells and ECM components. Plasmid DNA was delivered by PE-Gylated, cationic poly(*N*-substituted asparagines) micelles, and the PEG shielding in this study improved the cytotoxicity and particle penetration into hepatoma spheroids.[51] PEG shielding of a polyethyleneime (PEI)-based siRNA delivery sys tem also facilitated the delivery of siRNA through multicellular layers while helping the siRNA to maintain its integrity and activity within the 3D culture environment.[52]

In addition to charge shielding, PEG is advantageous for imparting amphiphilic properties onto hydrophobic polymers. The improved penetration of a PEGylated diblock copolymer micelle into a porcine bladder tissue was attributed to the improved partitioning of paclitaxel or doxorubicin into tissue due to the surfactant-like nature of the PEGylated copolymer delivery system.[116]

Another important parameter in the tumor transport properties of polymeric micelles is their particle size. As previously discussed with liposomes, small micelles are generally thought to diffuse more efficiently through a solid tumor matrix. As such, the efficient tumor penetration of paclitaxel from a dendridic polymeric micelle in a solid tumor model *in vivo* was attributed to their small size (20–60 nm).[117] The tumor penetration capabilities of PEG-block-poly(ε-caprolactone) micelles of 25 nm were compared with those of 60 nm diameter, and it was also found that the smaller particles had a more homogeneous *in vivo* tumor distribution.[118]

The incorporation of tumor-targeting ligands into polymer micelle systems has been studied for its effect on tumor penetration, yielding mixed results that are strongly system dependent. Two studies using epidermal growth factor (EGF) as a tumor-targeting ligand suggested that EGF might actually impede the tumor penetration of polymeric micelles due to its strong binding affinity to tumor cells.[118,119] On the other hand, the conjugation of lactose to a polymeric micelle system improved its spheroid penetration capabilities,[48] demonstrating that a particular targeting ligand should be screened for its influence on the tumor distribution of its drug cargo.

D. Inorganic Metal Nanoparticles

Inorganic nanoparticles have been widely used for drug delivery and imaging applications in solid tumors. For instance, magnetic nanoparticles can be used as MRI contrast agents, or to facilitate spatial control of nanoparticle location within tumors with an external magnet.[120] Gold nanoparticles have also been used in a variety of advanced drug delivery and diagnostic applications for the treatment of solid tumors.[121] High-resolution MR imaging was achieved by incorporating magnetic nanoparticles (~5 nm) into a dermatan carrier that was shown to achieve deep matrix penetration *in vivo.*[122] Metalbased nanoparticles can also be designed to improve the tumor-penetration capabilities of smallmolecule chemotherapeutic drugs, specifically by taking advantage of their magnetic properties. Magnetic nanoparticles have been used to improve the tumor penetration capabilities of camptothecin, and to enable its on-demand drug release from a polymeric particle shell.[123] A low-frequency external magnetic field was applied to guide movement of the particles to promote favorable distribution throughout the tumor tissue, and a radio frequency field was applied to heat the particles, promoting the active diffusion of camptothecin from the nanoshells. The unique properties of magnetic nanoparticles allow for this innovative approach to on-demand drug positioning and release.

The properties of inorganic nanoparticles have impacted their tumor penetration capabilities. The tumor accumulation *in vivo* of PEGylated gold nanoparticles was evaluated as a function of particle size, and like other materials smaller particles (20 nm), they showed more efficient tumor uptake and vessel extravasation than larger particles (40 or 80 nm).[124] However, in another study, larger iron oxide nanoparticles (40 nm) actually exhibited higher accumulation in squamous cell carcinoma tumors than smaller particles (20 nm).[125] In this case, the greater tumor accumulation by larger particles was attributed to their more efficient capture by tumor macrophages. This demonstrates the importance of designing nanoscale therapeutic agents for a specific tumor type and considering both physical and biological factors: size-dependent trends can change depending on the tumor context. In addition to tuning particle size, incorporating an ECM-degrading enzyme, collagenase, was shown to

improve the mobility of magnetic nanoparticles through an ECM-mimetic gel.[126] This approach may be extended to improving the penetration of magnetic nanoparticles through solid tumors.

E. Natural Biopolymeric Nanoparticles

Biodegradable protein nanoparticles have found applications in drug and gene delivery[127] due to their ability to encapsulate drugs of varying hydrophobicity and to their inherently biodegradable composition. Similar to other nanoparticles, protein particles have exhibited limited penetration into solid tumors. In a recent study utilizing gelatin nanoparticles to deliver cisplatin to murine hepatic tumors *in vivo,* the nanoparticles only delivered the drug to cells near the tumor vasculature.[128] Some approaches have improved the tumoral penetration of protein nanoparticles. A tumor-homing peptide called LyP-1 was used to target the interstitial tissue of tumors and blood vessel walls. When conjugated to the albumin-based nanoparticle, abraxane, LyP-1 facilitated the extravascular localization of the nanoparticles in breast cancer tumor xenografts *in vivo.*[129] Another very innovative approach utilized the biodegradation properties of gelatin to improve tumor penetration of particles in a multistage delivery approach.[130] Gelatin nanoparticles of 100 nm were formulated to encapsulate smaller 10-nm quantum dot nanoparticles. The larger gelatin nanoparticles accumulated in the tumor vasculature due to the enhanced permeability and retention effect. Once inside the tumor interstitium, the gelatin was degraded in the presence of tumor proteases, releasing the smaller 10 nm particles, which readily diffused through the tumor interstitial space. This strategy may provide a practical and powerful solution for protein nanoparticles to elicit deep penetration of drug cargo within solid tumors.

F. Polycations for Gene Delivery

Polycationic polymers have been widely used to deliver genes and antisense molecules to solid tumors by forming electrostatic nanocomplexes with anionic nucleic acid molecules.[131] Because it is necessary to maintain a net positive charge on nucleic acid delivery vehicles,[132] these cationic complexes have been largely unable to penetrate into solid tumor models in part because they undergo electrostatic association with anionic charged cells or ECM proteins. A widely used gene-delivery polymer, polyethyleneimine (PEI), exhibited poor tissue penetration when delivering plasmid DNA to tumor spheroids.[50] In other cases, cationic polymers that successfully facilitated gene transfection in traditional monolayer cell culture were significantly less efficient in delivering genes in a 3D tumor model due to poor tissue penetration of the charged complexes.[49,133] One approach used by our group to improve the tumor penetration of siRNA delivered by polyamidoamine (PAMAM) dendrimers was to attach an integrin-targeting peptide RGD ligand to the dendrimer surface.[47] The presence of RGD peptides improved the penetration of siRNA through tumor spheroids, presumably by interfering with the tight cell-ECM interaction present in the solid tumor model. An analogue of conventional RGD peptides, iRGD, has recently been developed to improve the tumor penetration of anticancer agents by enabling integrin-mediated cellular binding followed by neuropilin-1-based internalization of drug cargo.[134,135] Such promising RGD-based drug delivery strategies may find utility for enabling the delivery of nanoscale therapeutics, including nucleic acids, which are located homogeneously throughout solid tumors.

G. Tumor Microenvironmental Modulation

In addition to modifying the architecture of nanoparticles to alter their tumoral distribution, another successful approach has been to modify the tumor microenvironment to enhance drug uptake and penetration.[136,137] The tumor microenvironment is not “permissive” to drug transport due to an abnormally high ECM density that inhibits the successful uptake of therapeutic particles. This restrictive microenvironment can be “normalized” by the addition

of agents that interfere with vascular growth factors (e.g., VEGF, PDGF, and TGF-β) or agents to degrade components of the ECM. These strategies tend to "normalize" the pressure gradients across the tumor vasculature, and they may enhance the extravasation of anticancer therapeutics, including nanoparticles. The addition of matrix modifiers including collagenase,[22] relaxin,[138,139] and matrix metalloproteinases[23] have improved the efficacy of anticancer viruses by modifying the tumor microenvironment. A related approach is to blockade the VEGF-receptor-, which led to decreased tumor IFP and vascular normalization accompanied by improved vascular extravasation and deeper tumor penetration of a macromolecular protein, bovine serum albumin (BSA), in a murine mammary carcinoma model.[15] Similar normalization approaches have also proven successful in enhancing the delivery of larger, nanoscale therapeutics in solid tumors. Pretreatment of tumor spheroids with collagenase improved the penetration of nanoparticles through the *in vitro* tumor model.[19] In a recent, innovative, approach to modulating the tumoral ECM, the FDA-approved drug losartan decreased the production of tumoral collagen, enhancing the distribution and anticancer efficacy of Doxil in several *in vivo* murine tumor models.[24] The ability to modulate the tumor microenvironment may provide a powerful approach to improving the efficacy of nanoscale anticancer agents because it can be used in conjunction with nanoparticles of optimized chemistry and architecture.

VI. SUMMARY AND FUTURE RESEARCH DIRECTIONS

Several classes of nanoscale materials exist that are useful for drug delivery to solid tumors, each with unique properties making them practical for a particular application. For example, while liposomal or polymeric nanoparticles are practical for delivering small-molecule drugs and nucleic acids, the magnetic properties of inorganic metal nanoparticles can be exploited for imaging deep within tumor tissue. For most applications, the ability to penetrate deep within solid tumor tissue is necessary to elicit efficacious anticancer therapy. Approaches to studying tumor penetration and distribution of nanoscale materials have been described including *in vitro* cell culture tumor models, theoretical modeling techniques, and *in vivo* tumor imaging. These techniques enable researchers to visualize and understand better the mechanisms governing nanoparticle distribution in solid tumors. The influence of nanoparticle design parameters was reviewed for various nanoparticle types; several nanoparticle design parameters have been found to universally influence tumor penetration, regardless of the delivery system utilized.

The size of nanoparticles was identified as a critical design parameter governing their tumor penetration abilities. Because tumor transport is a diffusion-limited process, nanoparticles of a smaller size (≤20 nm) are found almost universally to diffuse more efficiently through tumor tissue than particles of a larger size (~100 nm or greater). Experimental demonstration of the strong relationship between particle size and tumor diffusion is shown in Figure 3. To the extent that it is possible to tune the size of particles formed by liposomes, synthetic polymers, and proteins, size optimization should be performed universally when designing nanomaterials for tumoral drug delivery. The innovative approach recently employed by Wong et al.,[130] whereby large 100-nm particles were engineered to release 10-nm particles once inside the tumor environment, could be useful to other researchers because this technique enables passive tumor-targeting by the EPR effect of large particles followed by efficient tumor penetration of smaller cargo particles.

The surface charge of nanoparticles is also an important parameter governing their tumor penetration and distribution. The surface charge of liposomes, in particular, has been carefully studied, and it was found that slightly cationic liposomes generally exhibit the most favorable tumor distribution. The surface charge of many classes of nanoparticles can be controlled by employing surface PEGylation. The presence of hydrophilic PEG chains on

the surface of a particle can shield the particle, reducing its electrosatic interactions without eliminating the charge, which may be necessary, particularly for nucleic acid delivery. Grafting varying lengths or densities of PEG cancbe performed on a variety of nanoscale carriers including liposomes, synthetic polymers, protein particles, or inorganic nanoparticles, and this technique may be a useful way for researchers to optimize particle charge for improved tumor penetration capabilities.

The presence of tumor-targeting ligands has been evaluated for its impact on the tumoral distribution of nanoscale materials, with mixed results. Tumor-targeting ligands are well known to improve the *in vivo* efficacy of drug delivery systems. However, their role in drug biodistribution and tumor distribution remains relatively unexplored.[140] In this review, several examples were presented in which the presence of a targeting ligand was evaluated for impacting tumor penetration capabilities of a nanoparticle drug carrier. Mixed results were obtained; some targeting ligands such as EGF impeded the tumoral penetration of drug carriers,[118,119] and other ligands such as RGD peptides, [47,135] iRGD peptides,[135] or lactose[48] improved the tumoral distribution of nanoparticles. Because targeting ligands are important in localizing drug carriers to malignant tumors, it is necessary for researchers to also study the presence of targeting ligands on drug penetration and distribution within the tumor following its initial accumulation. The type of ligand as well as its valency from a nanoparticle scaffold should be optimized by researchers to elicit favorable tumor-penetration properties.

Another approach used to improve the tumor penetration of nanoscale materials is to modulate the tumor ECM. The presence of an abnormally dense ECM in solid tumors inhibits the penetration of anticancer drugs.[20] Degradation of some ECM proteins, including collagen and hyaluronan, by the incorporation of ECM-degrading enzymes has enhanced the penetration of drugs[22,23] or nanoparticles[19] through solid tumors. However, because the use of these enzymes (e.g., collagenase) is currently not FDA-approved for the treatment of cancer in humans, it may be advantageous to find a more practical method to target the ECM. The use of the FDA-approved drug, losartan, represents one such approach.[24] The administration of losartan decreased the collagen production in solid tumors and enhanced the efficacy of the liposomal formulation, Doxil *in vivo*. This innovative strategy may provide a clinically translatable solution to normalizing the tumor vasculature for drug delivery purposes. RGD peptides have also found utility in improving drug penetration into solid tumors due to their ability to interfere with the interactions between tumor cells and ECM proteins. [47,135] RGD peptides have been used clinically for the treatment of cancer,[141] and they should be further explored and optimized for their additional ability to improve the tumor penetration of nanoscale therapeutics.

In addition to directly modifying nanoparticle systems via chemical crosslinking, other approaches can be applied to nanomaterials of any chemistry. Strategies including sequential dosing, [108] administering pulsed ultrasound,[53] employing hyperthermia,[109–111] or utilizing convection enhanced delivery[142] can be used in conjunction with nanoparticles of optimized chemistry to further improve their distribution in tumors.

VII. CONCLUSIONS

The use of nanomaterials for drug delivery and imaging of solid tumors holds significant promise for the treatment of human disease. However, poor penetration of nanoscale therapeutics into solid tumors hinders their anticancer efficacy. The work reviewed here demonstrates that particle design parameters are critical to achieve favorable tumor penetration and distribution. While some parameters including particle surface charge and the presence of targeting ligands have yielded mixed results, the effect of particle size is

indisputable. Particles larger than 100 nm universally do not distribute well throughout tumor tissue, regardless of other characteristics. However, simply using small nanoparticles is not sufficient to ensure favorable tumor distribution. Small nanoparticles do not accumulate in the tumor vasculature by the EPR effect, and they do not necessarily achieve good tumor penetration depending on their other physical properties. Thus, it is necessary to simultaneously optimize many particle design parameters (e.g., size, charge, and targeting groups) to ensure tumor tissue distribution. The benefits of employing physical stimuli such as magnetic fields, ultrasound, or convection-enhanced delivery have also been demonstrated here, and the importance of modulating the tumor microenvironment by vascular normalization or by ECM degradation has been highlighted. Future anticancer therapeutics will likely incorporate optimized particle chemistries with a physical stimulus to guide particle location in tumors. This multipronged design approach should yield improved anticancer therapies that may lower the necessary drug dose to patients, and it may produce novel techniques to improve the imaging of tumors *in vivo*. Designing nanoparticle therapeutics to improve tumor penetration may represent an important avenue to improve their *in vivo* efficacy.

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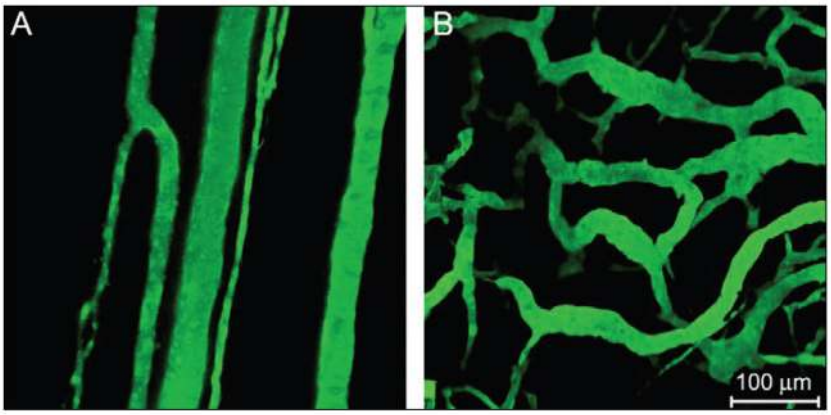

FIGURE 1.

Micrographs of normal (A) and tumor (B) vasculature acquired from nude mice bearing tumors from human squamous cell carcinoma cells. This figure was reproduced from: Dreher MR et al.[143] with permission from Oxford University Press.

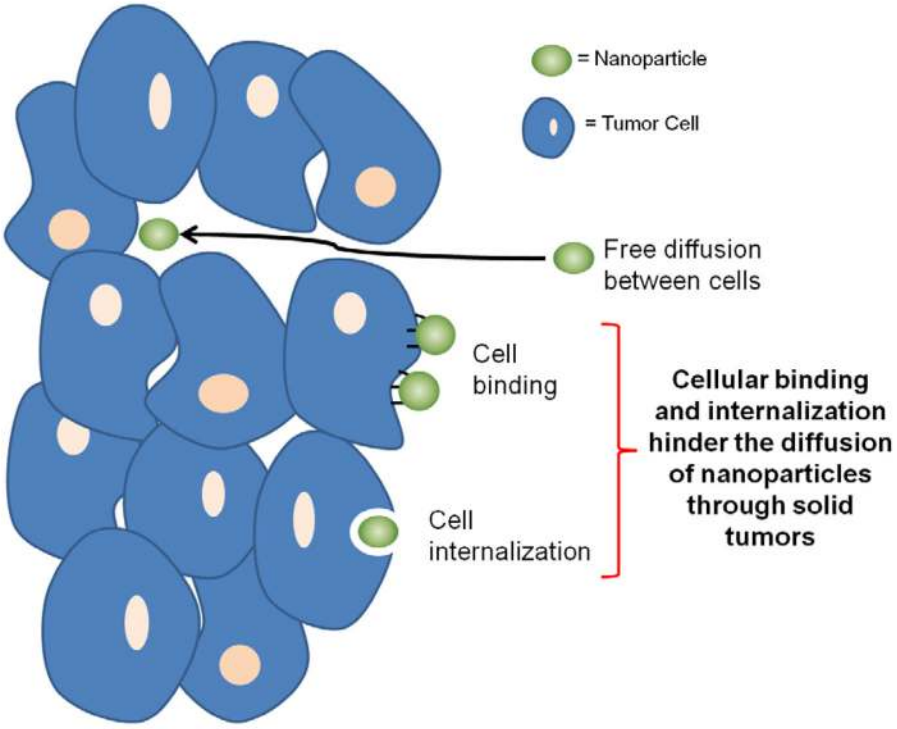

FIGURE 2.
Transport mechanisms governing nanoparticle penetration through solid tumors. Nanoparticles are transported through tumors by free diffusion in extracellular space which can be inhibited by cell binding and/or by cell internalization.

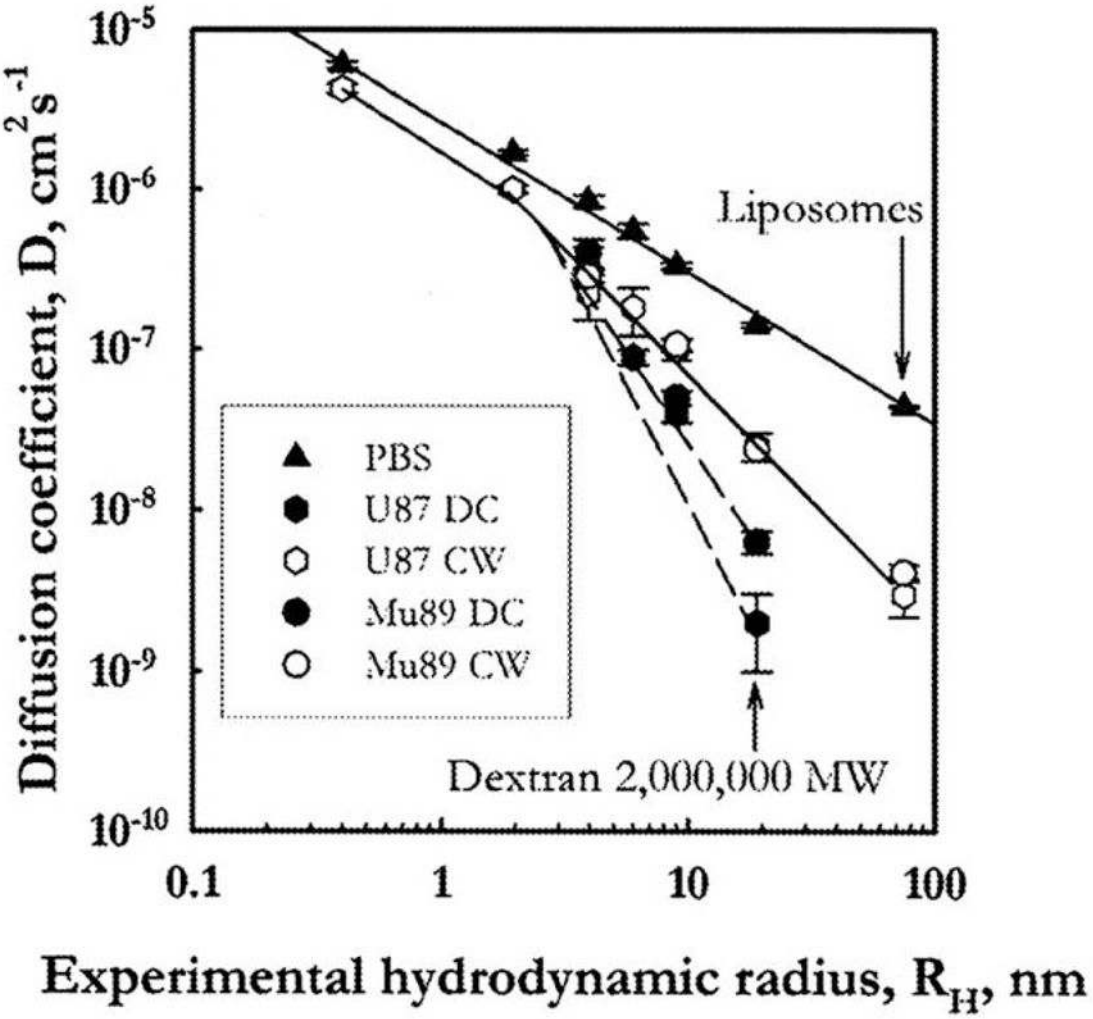

FIGURE 3.

Effective diffusion coefficients of macromolecules as a function of hydrodynamic radius in dorsal chamber (DC) and cranial window (CW) tumors. This figure is reproduced from Pluen et al.[16] Copyright held by the National Academy of Sciences.