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.::VOLUME 14 (XIV), LESSON 7::.

Nanotechnology in Nuclear Medicine

Continuing Education for Nuclear Pharmacists
And
Nuclear Medicine Professionals

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Nanotechnology in Nuclear Medicine

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NANOTECHNOLOGY IN NUCLEAR MEDICINE

STATEMENT OF LEARNING OBJECTIVES:

The purpose of this lesson is to provide a review of the ways that nanotechnology is currently implemented in medical applications and the role that nuclear medicine plays within those applications. Further, a few examples are presented on radiolabeling various nanocarriers for PET imaging and the feedback it provides on the design of nanocarrier systems. In this case, nuclear imaging serves as an assessment tool for designing new therapies and contrast agent formulations for imaging.

Upon successful completion of this lesson, the reader should be able to:

1. Describe nanotechnology advances in medicine and its impact on applications for both therapy and diagnostic imaging.
2. Understand the requirements for designing a nanoparticle to carry a radioisotope for therapy or imaging purposes.
3. Describe the design requirements for an imaging strategy, including the appropriate label and imaging modality for the task.
4. Understand the physiologic processes that may alter the biodistribution of various labeled probes.
5. Discuss the barriers that nanotechnology must overcome for cancer treatment and detection.
6. Define the limitations of various image analysis techniques.

COURSE OUTLINE

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NANOTECHNOLOGY IN NUCLEAR MEDICINE

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INTRODUCTION

Nanotechnology is loosely defined as systems that are less than 1 micron in diameter. In nanomedicine, these are organic and inorganic nanoparticles or nanocarriers that aid in non-invasive imaging and therapy of various pathologies (1). The major application of nanotechnology in medicine is cancer therapy and detection, sometimes termed "theranostics" in which nanoparticles overcome biological barriers to improve detection, diagnostics, treatment, and can even monitor disease progression and therapeutic outcomes, in some cases using the same nanocarrier.

The majority of this module reviews the challenges surrounding tumor detection and therapy and the ways that nanotechnology and nuclear imaging are combined in the research realm to overcome these barriers. Goals in this area include creating novel particles and strategies for therapy, for nuclear imaging and diagnostics, and for nuclear imaging techniques that aid in the design of these novel particles and delivery systems. The later portion focuses on a positron emission tomography imaging scheme that provides valuable feedback in the design of lipid based-particles for drug delivery.

CHALLENGES FOR NANOTECHNOLOGY: TARGETING THERAPY TO TUMORS

In directing therapeutic molecules to tumors, it has been determined that three major limiting factors contribute challenges that nanotechnology must overcome on a systematic scale. The first of these factors is an uneven distribution of a drug molecule in the organs of the body, where kidney and liver have the most concentrated levels of drug because they are highly vascular organs. Secondly, small molecules are readily excreted, and therefore have a short circulation times. The third factor is drug inactivation by irreversible binding to proteins, while larger particles are retained in the spleen (2). The tumor itself provides many hurdles. The largest challenge is presented by the spatial and temporal heterogeneity of tumor vasculature (3, 4); this includes vessel diameter, length, permeability, integrin expression, density, and spatial distribution. Some of these attributes are tumor size dependent, such

as the necrotic cores of tumors that result from high interstitial pressure and lack adequate vasculature. While increased blood flow to tumors serves as a diagnostic marker in nuclear imaging (5), blood flow within a tumor is dependent on the vessel network, pressure, and blood viscosity and overall is disorganized and lower than other tissues. Tumor vasculature is tortuous and unpredictable, and as a consequence, it is hard to develop therapeutic strategies. A recent approach to improve therapeutic delivery is priming the tumor tissue with a course of treatment that changes tumor properties, such as interstitial pressure, to favor diffusion and convection to carry the therapeutic payload to its destination (3). While this notion seems counter-intuitive, a pretreatment of anti-angiogenic therapy or nutrient gradient based therapy was designed to improve or normalize blood flow and hence convective transport of a drug into a tumor. This technique has been used with some success, which is attributed to a pruning effect where inefficient and immature blood vessels are shut down leaving behind the relatively mature vessels to flourish and become more efficient in transporting molecules into the tumor (6-8).

Mathematical models (3, 9) provide relationships between various tumor parameters and therapeutic delivery to solid tumors. Among the modeled attributes of tumors that contribute to the challenges in delivery are limited diffusion and convection. Tumor size also plays a role in the outcome of various treatments (10). It is more difficult to treat large tumors due to the increased interstitial pressure and lack of vasculature in the necrotic core. The increased interstitial fluid pressure resulting from poor lymphatic clearance hinders extravasation, as well as hindering further transport into the interstitium. In some cases, this can also be an advantage, as tumors retain macromolecules since lymphatic clearance of interstitial fluid is missing (2). To conclude upon the many challenges in delivering molecules and particles to tumors, the dynamic nature of tumors greatly affect the effectiveness of therapy and should be assessed when designing schedules for treatment.

PARTICLES

Lipid-Based Nanocarriers

The so-called pharmaceutical “magic bullet” is a vehicle capable of delivering, yet, limiting treatment of a therapeutic molecule to a specific target (11). There is a plethora of diverse and important parameters in developing a drug delivery system with this ability, whether the vehicle is a liposome, micelle, microsphere or polymeric drug complex. A few of these factors are stability, size, charge, solubility, composition and drug-to-carrier ratio (12). If the drug to carrier ratio is low, potency of the drug to be delivered must be high to avoid carrier toxicity. In general, the pharmacokinetic profile of a

drug-carrier complex is between that of the free drug and the carrier profile. If the drug is released slowly, the overall profile is close to that of the carrier, while approaching that of the free drug when the drug is released rapidly (13). Lipid based complexes composed of high-phase-transition lipids, cholesterols, and glycolipids create a slightly negative surface charge, protecting against opsonization and aiding in increasing circulation time (10, 14-18). Figure 1 provides illustrations of various lipid based particles. Positively-charged vehicles tend to be cleared rapidly by the lungs, spleen, and liver making them attractive for delivery in those organs (19) and unattractive for tumor targeting. Size plays a significant role in biodistribution; larger sized particles are restricted to the vascular compartment, which increases the half-life and the area under the concentration-versus-time curve, decreases clearance of the drug, and decreases volume of distribution (10, 12, 20). However, the spleen filters out particulate drug delivery systems that are larger than 200 nm in diameter. This is not a strict rule, as a vehicle's ability to deform will aid in transport through this filter (21).

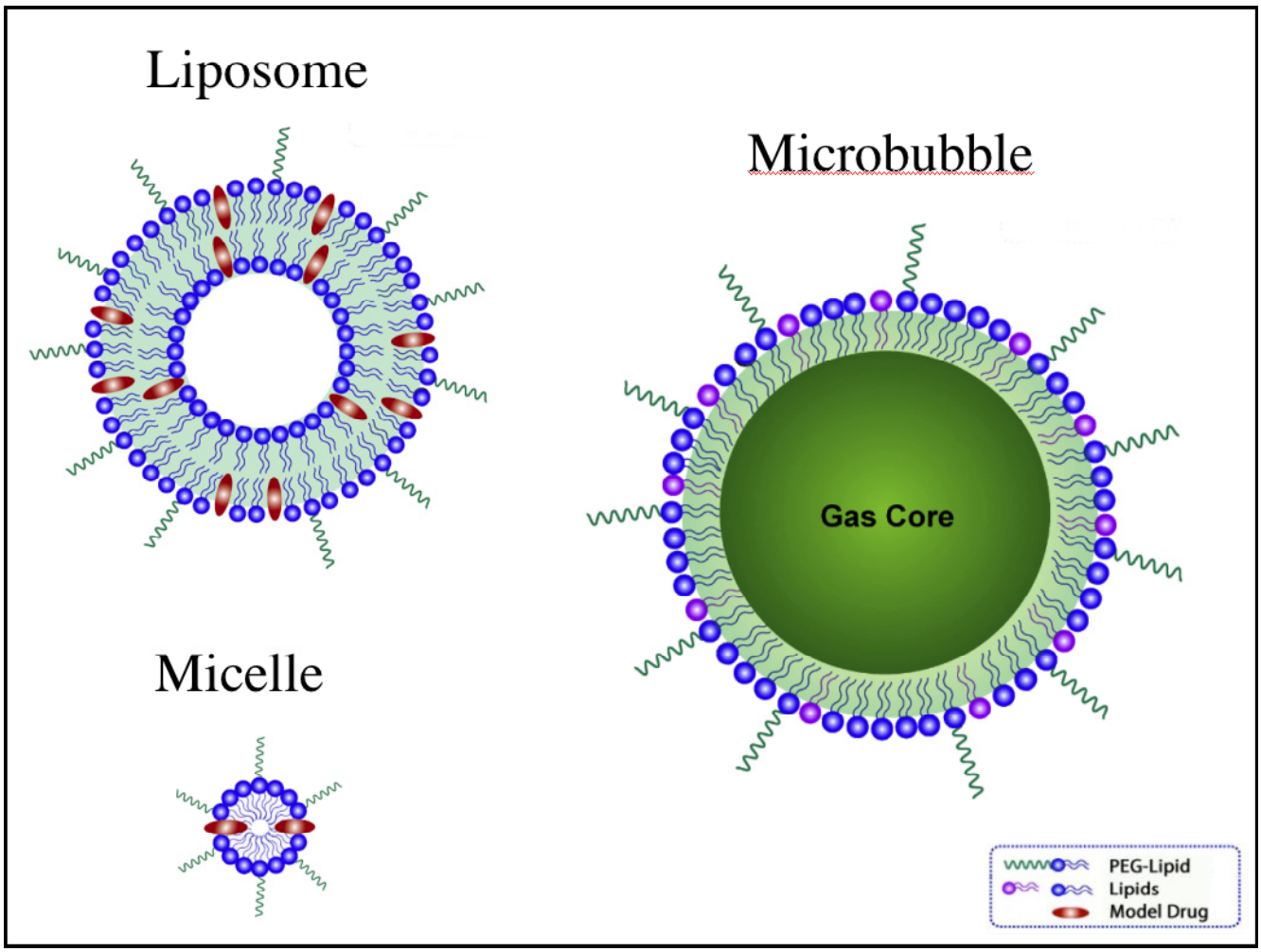

Figure 1. Illustration of various lipid based particles. The microbubble, liposome, and micelle are not drawn to scale. In the case of the micrcobubble, a model drug or genetic material may be associated with the lipid shell.

Liposomes

Liposomes are used as drug carriers because they are composed of biologically inert materials and are therefore non-toxic. Similar to cell membranes, they are vesicles made of self-assembled lipid bilayers. They have the ability to carry and protect a drug of interest from the external environment. Practically any drug can be incorporated into a liposome. Hydrophilic drugs are carried in the aqueous interior of a liposome, while hydrophobic and amphipathic molecules associate with the fatty acyl chains of the bilayer (11). The volume of aqueous media encapsulated in liposomes is much larger than that of the hydrophobic environment, making hydrophilic drug payload inherently larger; however, drug potency also affects the overall efficacy of therapy. Hydrophobic drugs with low solubility in blood are more susceptible to release from the vehicle to cell membranes, plasma, and proteins (12, 22). Delivery by liposomes also eliminates the need for using the highly toxic solubilizing agents often needed for delivering hydrophobic materials (23).

Currently, there are many successful methods for drug entrapment in liposomes. Some of these methods are extrusion, sonication, detergent dialysis and reverse-phase evaporation (11). The extrusion process using polycarbonate filters of various pore sizes allows control of the upper size limit of liposomes, which is useful when designing particles for long circulation and targeted applications. Liposomes can be endocytosed or fused to cells yielding the ability to effectively deliver drugs across the cell membrane. To achieve maximum localized delivery, the liposome must have high efficiency for entrapment of the drug into the liposome, prevent uptake by the reticuloendothelial system (RES), and have affinity for the target. One way to prevent RES clearance and increase the circulation time is through coating the liposomes with a variety of materials (10, 11).

Polyethylene glycol (PEG) coating of the liposome was the breakthrough leading to second-generation liposomes. PEG increases the hydrophilicity of the liposomes by concentrating hydrating groups on the surface, making it more in soluble aqueous fluids. This sterically hinders electrostatic and hydrophobic interactions with blood components, thereby reducing protein binding and opsonization (10, 24, 25). Numerous other surface modifications and liposome coatings have been examined to provide a hydrophilic sheath that would protect hydrophobic moieties of drug delivery vehicles from protein and enzymatic interactions, including biocompatible polymers such as, poly(ethylene oxide) (PEO), poly(propylene oxide) (PPO), polyesters, and their derivatives (26). Long circulation times introduce the issue of drug release from the liposome. High diffusion rates across the liposome bilayer are a concern; however, the drug must diffuse before the liposome clears the target tissue (2).

Micelles

Micelles are self-assembling structures composed of lipids or polymers, somewhat similar to liposomes, however, they are spherical aggregates and not lipid bilayers. The typical size range for micelles is on the order of tens of nanometers in diameter. In an aqueous environment, the hydrophobic moieties mingle in the core of the structure and polar head groups are in contact with the aqueous environment. This structure can accommodate hydrophobic and amphipathic molecules (12, 21, 27, 28). Examples of micelles used in conjunction with active or extracorporeal targeting are discussed in more detail in following sections.

Microbubbles

As the name signifies, microbubble contrast agents are on a different size scale, where lipid-based microbubbles can range from sub-micron to tens of microns. It is size that limits their biodistribution to the blood pool while intact. Microbubbles act as ultrasound contrast agents, increasing the acoustic signal reflected from blood. The increased signal arises because they are gas filled, making them lower in density and highly compressible compared with surrounding blood. Microbubbles oscillate spherically and nonlinearly when exposed to acoustic pressure waves because of these properties, making imaging and detection a highly sensitive modality. Microbubbles have been successful with gene delivery, but have had limited success with drug delivery due to the lack of space and adequate environment to carry large amounts of drug. Gene delivery with microbubbles has been successful due to the fact that plasmids and other genetic vectors can be associated with the microbubble (29) and consequently combined with cavitation induced by ultrasound application.

In vivo delivery of genes using microbubbles as a carrier has been demonstrated with and without ultrasound. Antisense phosphorodiamidate morpholino (PMO) has been delivered to endothelial cells *in vivo* using albumin-coated microbubbles, which bind to sites of vascular injury (30). Successful gene delivery to the myocardium with ultrasound-targeted microbubble destruction has also been demonstrated, where microbubbles had hVEGF plasmid-containing liposomes attached to the phospholipid shell (31).

Microbubble hybrid vehicles, called lipospheres (12, 32-35), have both targeting ligands and a thick drug-carrying oil layer and can be combined with ultrasound radiation force pulses to enhance local drug deposition on tumor endothelium (36, 37). Other ultrasound mediated drug delivery mechanisms

are also possible (38-40) and will be discussed with other modalities in combination with nanocarriers. However, this method addresses the specific problem of drug loading on a microbubble-based vehicle.

Nanocarriers for Hydrophobic Drugs

Initial liposomal formulations containing hydrophobic chemotherapeutics eliminated acute toxicity and increased the maximum tolerated dose. Despite the fact drugs released from the liposomes were protein bound as in the 'free drug' case, differences in pharmacokinetic parameters and biodistribution were observed (41). Stable hydrophobic incorporation into a vehicle is difficult to achieve. While hydrophobic drugs spontaneously associate with lipids in aqueous solutions, solubility is highly dependant on enthalpic energy and hence temperature (42, 43). Hydrophobic drug association with liposomes can also be reversible (44) and facilitate exchange between liposomes (43). Theories describing various drug association with the lipid bilayer hypothesize either full insertion into the bilayer or insertion in the outer leaflet of the bilayer only. If a drug resides in the outer leaflet alone, only one layer of lipid is perturbed or disrupted by its presence, resulting in a lower energy state than that of the fully inserted model, making the outer leaflet position the favored theory. A variety of factors affect entropic and enthapic barriers to drug incorporation in the bilayer and the length of time that a drug remains with the lipid bilayer including lipid composition, drug concentration, and the hydrophilic environment surrounding liposomes (43).

While both PEG and cholesterol have been shown to increase stability and circulation time of liposomes, they may hinder drug incorporation (23, 45). Cholesterol, specifically, is accommodated in regions of the bilayer where drug may reside and therefore lowers the amount of space available to accomodate other molecules. Cholesterol also restricts the movement of the hydrophobic chains, making the bilayer less accommodating. Finding a liposome that has both high encapsulation efficiency and high stability for hydrophobic molecules proves to be a balancing act in the *in vitro* setting. This leaves challenges in the *in vivo* setting to be addressed, where drug disociates from liposomes and may partitions to serum proteins (46).

PASSIVE TARGETING

A concept called the enhanced permeability and retention (EPR) effect can be exploited to increase delivery in tumors (21, 47). Increased permeability of tumor vasculature allows larger molecules and particles to diffuse into the surrounding tissue due to inter-endothelial fenestrations, the discontinuous basement membrane, and an increased rate of trans-endothelial transport that takes place in tumor

vasculature. Particles that depend on the EPR effect to reach the target site must have a long circulation time. Longevity of circulation is of utmost importance in this mechanism, as longer circulation time increases the passage of the drug-carrier complex in the tumor vasculature, thereby increasing the opportunity to cross the blood vessel wall into the tumor interstitium (10). Longer circulation times can be achieved by increasing the size of a drug-carrier complex from the small molecule range. This passive method of drug delivery is limited to tumors that have their own neovasculature, as opposed to those that feed off neighboring tissue (2). Passive targeting can be modeled (48) to predict the transport limitations of convection, diffusion and attainable drug concentrations. Simulations suggest that a more active targeting mechanism in combination with EPR effect would improve overall effectiveness.

ACTIVE TARGETING

Active targeting on the molecular level involves increasing affinity and specificity for the target tissue through cell surface receptors or molecular changes that are triggered by some chemical reaction or physical parameter specific to the target site. Examples include pro-drugs that are cleaved within a tumor; antibody and peptide conjugation to a vehicle surface or the drug itself; and pH-, oxygen- or temperature-sensitive vehicles.

pH-sensitive Polymers in Lipid Vehicles

Nanoparticles made of a biodegradable and pH-sensitive polymer, poly(ethylene oxide)-modified poly(β-amino ester), can carry hydrophobic drugs until arrival at the acidic microenvironment of tumors, or in endosomes and lysosomes of cells (26). This polymer also provides a hydrophilic sheath to increase circulation time, thereby taking advantage of the EPR effect. This particle releases its payload intracellularly at a higher rate than nanoparticles made of polymers that are not pH-sensitive, which remain intact after endocytosis. Once rapid pH-triggered release occurs and an effective drug concentration is reached, cytotoxicity predictably ensues, while nanoparticles behave somewhat unpredictably.

Peptide and Antibody Targeting for Lipid Vehicles

In order to increase specificity, targeting ligands are directly conjugated to the surface of imaging agents and drug delivery vehicles (49-56). Endothelial cell surface receptors can be highly expressed in disease states and can serve as imaging and therapeutic targets (57). An *in vivo* study used a

combination of antibodies to ICAM-1, VCAM-1, fibrin, fibrinogen, and tissue factors to target echogenic liposomes to atheromas for targeted ultrasound contrast enhancement (58).

Site-specific peptide sequences can be attached to vehicle surfaces. For instance, peptide sequences are capable of targeting the integrin $\alpha_v\beta_3$, which is over-expressed on the vascular endothelium of tumors. The integrin $\alpha_v\beta_3$ is highly expressed in this region due to the process of angiogenesis, which is the recruitment and growth of new blood vessels. Angiogenesis is a necessity for survival and growth of malignant tumors whose size is greater than $1mm^3$, making this an ideal target for both contrast imaging agents and chemotherapeutic drug delivery.

In studies where systemically injected nanoparticles targeted the $\alpha_v\beta_3$ integrin, therapeutic genes were successfully delivered to tumors in mice. These particles were lipid-based, using lipid composition and polymerization to achieve a ligand conjugated surface with varying charge. As a result, apoptosis of the vascular endothelium of the tumor, subsequent apoptosis of tumor cells, and finally tumor regression occurred (59). A bicyclic RGD analog was conjugated directly to a drug molecules, specifically the paclitaxel molecule (RGD-PTX) to increase the selective binding of the complex to the $\alpha_v\beta_3$ integrin expressed on both endothelial and malignant cells in tumor tissue (60). Selective uptake in the tumor tissue was observed 24 hours post-injection using an ^{125}I-labeled analog of RDG-PTX, while maintaining its cytotoxicity with a slight decrease in binding affinity for the target. The conjugated molecule imposes cell death in the M/G_2 phase. These results show promise for tumor retention of a compound resulting in lower systemic dose. However, future animal studies will reveal the efficacy of this method.

Microbubbles or ultrasound contrast agents (UCAs) can be targeted using antibodies or peptides attached to the lipid shell (61-64). *In vitro*, the adhesion of targeted UCAs to $\alpha_v\beta_3$-expressing cell lines was increased at least 20-fold over non-targeted UCAs (61). Targeted UCAs used a ligand based on arginine-glycine-aspartic acid (RGD). Selectivity was also demonstrated by observing inhibition of UCA binding after pre-incubation of the $\alpha_v\beta_3$ sites with free RGD peptide. It has also been demonstrated that ultrasound radiation force applied to targeted UCAs significantly increases the number of adherent UCAs (64).

EXTRACORPOREAL TARGETING

The use of external force acting on a target region within the body has been demonstrated for treatment of cancerous tumors, for instance, thermal ablation of tissues. Similar techniques have also been applied in conjunction with nanotechnology, such as inorganic, polymeric and lipid-based particles. This section surveys a few of the diverse technologies that incorporate nanocarriers and an extracorporeal method of activation, all of which can benefit from non-invasive imaging assessments of performance. In most cases, feedback is vital to asses the success of each strategy and often aids in the design.

Magnetic Based Approaches

Researchers have developed a ferrofluid that associates a chemotherapeutic with a magnetic nanoparticle (65). An external magnet is applied in the region of interest and held there for a prolonged period of time, such that the ferrofluid, and therefore drug, accumulates at the target region. Advantages associated with this approach include a highly localized treatment, minimal toxicity and invasiveness, and validation of delivery can be achieved non-invasively with magnetic resonance imaging (MRI). In addition to the observed minimal toxicity, tumor volume was drastically decreased within a month. Histology revealed 'nanomagnet' delivery to both tumor endothelium and interstitium. However, the disadvantages to this approach are not easily dismissed. Larger magnetic particles could potentially embolize, while the smaller particles have little magnetic force, rendering them ineffective. This method is also limited by depth of penetration, restricting its potential to superficial tumors as opposed to deeper masses. It is preferable to inject this formulation into the feeder vessels of the tumor to avoid a first pass effect. The efficacy of this approach, as with all particulate drug delivery systems, is dependent on the tumor microvasculature.

Other magnetic particles such as magneto-liposomes are also being used in a similar fashion (66). These lipid-based particles carry doxorubicin as well as a magnetic fluid. Deposition of the drug within the tissue was observed upon heating. Particles were also observed outside of the vasculature, presumably displaced by the force of the magnetic field. Hyperthermia alone, caused by the combination of a magnetic field and magnetic nanoparticles, also produces tumor growth arrest and has been demonstrated in the treatment of prostate cancer in rats (67).

Finally, magnetically targeted nanoparticles are also being used to deliver radioisotopes, such as ^{188}Re (68). Once again, ideally, these magnetic fields are used to act upon systemically administered

particles to enhance uptake in tumor tissue for a targeted radiotherapy regimen. These particles, composed of magnetite and coated with albumin, have achieved roughly 90% labeling efficiency and stability of 72 hours. These magnetite cores have also been coated with polymers or silica allowing them to be targeted with monoclonal antibodies *in vitro* (69).

Ultrasound Based Approaches

A variety of ultrasound mechanisms exist that can be used for drug delivery applications in tumors. A brief list includes: sonoporation, permeability enhancement, and radiation forces. As a therapeutic modality, high-intensity focused ultrasound (HIFU) has had much success as an extracorporeal and thermal ablation method to direct treatment to tumors (70-73). This section addresses various ultrasound mechanisms used specifically to enhance drug delivery in conjunction with nanocarriers.

Pre-clinically, micelles encapsulating the chemotherapeutic doxorubicin have been successfully used with ultrasound to enhance drug delivery. Decreased ovarian carcinoma tumor growth rates and increased mouse survival rates were observed after applying unfocused and continuous wave ultrasound (27). This unique mechanism relies on the polymeric micelles first accumulating in the tumor via the EPR effect before ultrasound application. This method increases cellular uptake rather than aiding the micelle across the endothelium. Upon inspection, drug deposition was more uniformly distributed in the tumor tissue once ultrasound was applied. Ultrasound thermal effects were ruled out after careful investigation of temperature change with ultrasound application and free drug uptake enhancement with temperature change. Another parameter impacting the efficacy of this method is frequency, where lower frequencies (1 MHz) are more penetrating, potentially expanding past the boundaries for intended treatment, and higher frequencies (10 MHz) are rapidly attenuated, potentially disabling treatment from reaching a target. Again treatment was unsuccessful when treatment commenced after the tumor reached a threshold size, as with many treatment strategies.

Enhanced vascular permeability with high-intensity focused ultrasound (HIFU) increases the percentage of fluorescently-labeled polystyrene nanoparticles deposited in tumor interstitium above control contralateral tumors *in vivo* (74, 75). This suggests an increased therapeutic effect due to increased drug-containing particles in the tumor. However, when doxorubicin-containing liposomes with the same diameter as the nanoparticles were injected after HIFU exposure, there was neither increased drug concentration nor increased therapeutic effect. The lack of improved efficacy is thought to be a result of the ability of liposomes to extravasate in leaky tumor vasculature due to the

EPR effect, that is to say that ultrasound does not increase the already present extravasation. However, HIFU does seem to enhance gene delivery in tumors and consequent reporter gene expression (76). There is potential for HIFU to increase gene delivery in tumors specifically by combining HIFU with ultrasound contrast agents, which may produce cavitation and sonoporation (77).

Sonoporation of cell membranes has been researched as a possible avenue for directly inserting a desired drug into a cell (78, 79) or as a means to enhance permeability locally such that subsequent drug administration would preferentially accumulate at the site of sonotherapy (80-82). During sonoporation, ultrasound bioeffects cause the membrane itself to become temporarily permeable. These mechanical effects can be also detrimental to the cells being treated (83), preventing them from actively transporting drugs further into the tissue. Delivering a drug to a cell surface may be sufficient to cause a therapeutic effect by triggering a cascade of events starting with the binding of a drug to a cell surface receptor.

Low intensity ultrasound pulses are capable of bringing drug delivery particles into contact with cells, allowing the particle to enter the cell. An *in vitro* assessment of ultrasound mediated delivery to cells using liquid perfluorocarbon nanoparticles labeled with fluorescence demonstrated that lipophilic substances can be delivered directly to the cytoplasm after ultrasound induced fusion (84). Attaching targeting ligands for the $\alpha_v\beta_3$ integrin, which is highly expressed in tumor neovasculature, to the surface of the nanoparticle enhanced this effect. This study makes a case that non-cavitational ultrasound energy, radiation force, is capable of delivering drugs by the initiation of membrane fusion.

Ultrasound advantages for cancer therapy include both imaging and therapeutic capabilites providing diagnosis and monitoring of therapeutic progress. In addition to the variety of mechanisms that may be involved drug delivery with ultrasound, the advantages inherent with ultrasound are portability, relatively low cost, depth of penetration, real-time imaging, and noninvasive in nature.

Laser Based Approaches

Yet another technique is based on ‘nanoshells’ and photothermal mechanisms, where inorganic nanoparticles absorb energy from a laser at specific wavelengths to acheive thermal ablation (85-87). Nanoshells are composed of a thin outer gold shell and an inner core composed of silica. These particles also scatter light in the near infrared (NIR), making them ideal for imaging purposes making this scheme an analogue to the ultrasound methods, where ultrasound contrast agents can be

molecularly targeted and are used for both imaging and therapeutic purposes. This laser-based method has been investigated both *in vitro* and *in vivo* demonstrating that these particles can circulate on the order of 6 hours, that they can selectively ablate cells based on targeting ligands, and that this method has minimal sytemic toxicity and invasiveness. Again, with this method the limitations are the dependancy on tumor vasculature and the depth of penetration, which restricts treatment to superficial tumors. Attempting to treat deep tumors could significantly heat the tissue between the light source and the intended target. The nanoshells themselves can be transported across blood vessel walls and then remain in the body for an extended period of time.

NUCLEAR IMAGING TO DETECT AND ANALYZE PARTICLE TARGETING PERFORMANCE

Radionuclides have been widely used in imaging applications for single photon emission computed tomography (SPECT) and positron emission tomography (PET) and have been combined more recently with drug carriers to create novel imaging and therapeutic strategies for cancerous tumors. Additionally, combination therapies are gaining momentum, where either multiple chemotherapeutics are used or radiotherapy and chemotherapy are combined in one or more targeted nanoparticle. The need to validate biodistribution of drugs and delivery vehicles is not only useful in feedback for design of novel methods, but can also to predict patient response to the therapy (28, 88). A biodistribution or imaging study prior to drug administration might prevent the unnecessary exposure of those patients who might not benefit from a particular treatment (10, 88). The following material demonstrates successful *in vivo* tracking of therapeutics and therapeutic carriers with radiolabeling techniques for PET and SPECT imaging. There are many studies on radiolabeling inorganic nanoparticles, dendrimers, hydrogels, and other polymeric constructs in addition to the liposomes, micelles, and lipid-based nanoparticles focused on in this section (1). In general, these non-lipid based particles are often labeled with SPECT agents, while lipid based particles have been labeled with both PET and SPECT agents. Concerns for developing for radiolabeled particles include labeling efficiency, stability in plasma, long blood circulation time, and evasion of the RES. Imaging the distribution of these agents in each case has furthered knowledge regarding delivery strategies. Figure 2 provides an example of lipid based particles, where a portion of the lipid composing the particles have been radiolabeled with ^{18}F.

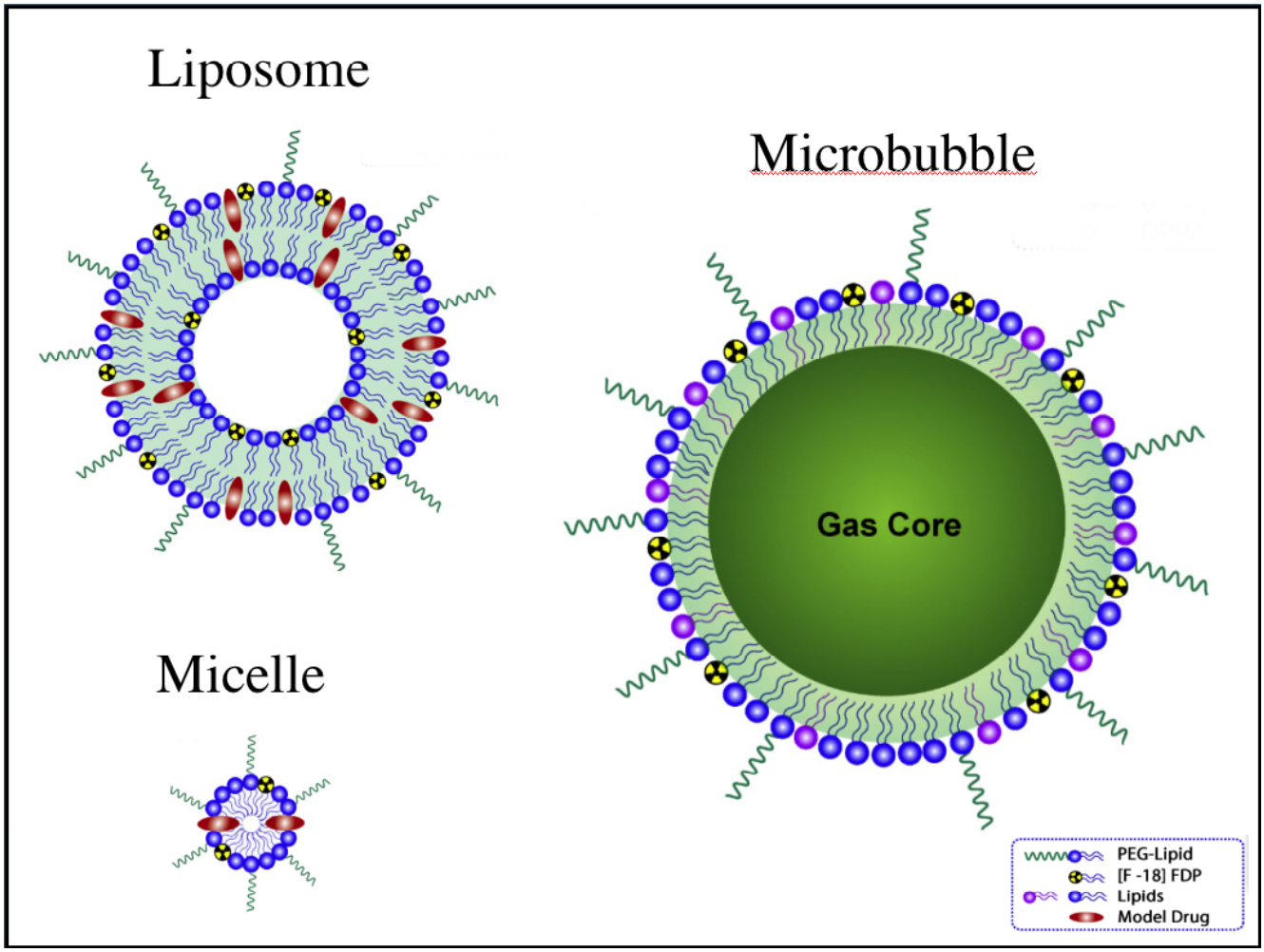

Figure 2. Illustration of radiolabeled lipid based particles. In this case the some of the lipids composing the various particles have been labeled with ^{18}F.

PET and SPECT are highly sensitive modalities for functional imaging, often complimented by anatomical information obtained from x-ray computed tomography in combined instruments such as PET/CT and SPECT/CT, both in the clinical setting for human diagnostics and in research setting with small animals (89-91). Briefly, the advantages of using PET or SPECT in nanotechnology applications are numerous and include high sensitivity, the ability to trace molecules over time, and the ability to non-invasively image a small set of animals multiple times thereby allowing each animal to serve as its own control.

A caveat that exists in any imaging modality and corresponding contrast enhancement agent is that chemical modification of a molecule of interest to include a tag or marker may change its functionality. When attaching a molecular tag to gain feedback, one must be cognizant of how that modification affects molecular function and consequently, the feedback itself. Additionally, PET imaging detects the radiolabel itself and does not distinguish between parent compounds and metabolites. Evaluations

are generally performed to assess the metabolism of a radiolabeled molecule after administration (92), which is particularly important when interested in pharmacokinetic parameters. These assays are performed to detect the presence of radiolabeled metabolites, which might skew biodistribution data. However, if radiolabeled metabolite presence and distribution is known, it can be accounted for and in some cases can be corrected (88).

Delivery of Gamma-Imaging Agents by Liposomes

Liposomes are used for both imaging and delivering therapy as they are capable of carrying large payloads and readily targeted using peptides and antibodies for various pathologies. Combining proper contrast-enhancing payloads and surface architecture allows liposomes to be readily used in diverse applications such as imaging blood pool, detecting inflammation, and treating tumors. A list of ideal characteristics specifically for liposomal gamma-imaging agents or SPECT agents has been developed. This list includes long shelf life, high labeling efficiency, ease of labeling with readily available radionuclides with reasonable half-lives and imaging quality, retention of radionuclide within the liposome, and the ablity to be inserted into a variety of liposomes (93). Several methods have been used for lipid-based particles where the radiolabel is either contained within the aqueous core of the liposome, on the surface of the liposome, or within the bilayer. A few brief examples are reviewed in this segment.

Despite undesirable characteristics, including high-energy emission and a half-life of 78 hours, direct labeling of liposomes was achieved with ^{67}Ga. The high energy requires thick lead shielding and limits the patient dose. Imaging of single photon emitters also exhibits lower resolution than PET imaging schemes, as a consequence of detecting single photons rather than photon pairs from coincident events. However, the half-life of ^{67}Ga does have the advantage of long tracking time of liposomes after administration. ^{67}Ga has been successfully chelated to nitriloacetic acid and encapsulated in the aqueous phase of liposomes, has albeit with poor labeling efficiency.

Direct incubation of ^{111}In with liposomes has been successful *in vitro* but is rather unstable *in vivo*. Incorporation of the chelator DTPA in to the lipid membrane of the liposomes allows for direct incubation with ^{111}In and provides stability *in vivo*. An additional step is required in this encapsulation method, in which the lipid mixture must be heated above their phase transition for maximum labeling.

Liposomes of this kind can be used in applications such as determination of drug distribution of encapsulated drugs. Many studies have already used radiolabeled liposomes to determine

biodistributions and have increased the amount of information per study with nuclear imaging as the distribution can be viewed at multiple time points.

PET Tracking of Liposomes and Nanoparticles

Insertion of [2-^{18}F]fluorodeoxyglucose ([2-^{18}F]FDG) into liposomes has been used to demonstrate tumor uptake of liposomes in imaging studies (94). With this liposomal tracking assay the effects of various liposome parameters on tumor uptake via passive targeting were observed. These parameters were liposome coating materials, size, and charge. Ultimately, these studies have led to the optimization of liposomes for imaging as well as targeted therapeutic delivery.

Studies using liposomes containing PET agents were made by freeze-thaw methods with liquid nitrogen to capture the radionuclide. The liposomes were sized by extrusion through polycarbonate filters with various cut off sizes. This study first validated that less than 10% of the radionuclide, [2-^{18}F]FDG, was released from the liposomes. Three kinds of lipid coatings, polyethylene glycol (PEG), gangioside (GM1), and palmityl glucuronide (PGlcUA) were tested against a conventional liposome formulation for longest circulation in the blood stream (and therefore most tumor uptake via passive targeting) and least accumulation in the RES. The results showed that the best coating for tumor uptake was the PGlcUA with PEG as a close second. All three coatings improved circulation time over the conventional formulation and less uptake by the organs of the RES, such as liver and spleen.

PGlcUA coated liposomes were used to study the effect of size on biodistribution. Four liposome diameters were tested: 100, 200, 300, and 400 nm (95). Tumor uptake was maximized at a liposome size of 100 nm. In the liver, 300 and 400 nm sized liposomes were taken up more than the 100 nm and 200 nm sized liposomes over time. The smaller liposomes (100 nm to 200 nm) were rapidly taken up in the liver, but then re-released into the bloodstream, while separate studies involving GM1 liposomes revealed that smaller sized liposomes (about 70 nm) (96), were rapidly taken up and retained. Spleen uptake was maximized with the 300 nm and 400 nm liposomes.

Charge was investigated by using positively charged, negatively charged, and neutrally charged liposomes (97). The tests performed were turbidity and serum binding assays. The positively charged liposomes created the most aggregates and were found to bind the most to serum. *In vivo* tests revealed that liver and spleen uptake was maximized with positively charged liposomes, while the negatively charged liposomes had an intermediate uptake in both organs and neutral liposome had

minimal uptake. Overall the best liposome for tumor uptake was the PGlcUA coated liposome of neutral charge and about 100 nm in diameter.

In vivo experiments where the hydrophobic drug, paclitaxel, was incorporated into pH-sensitive nanoparticles involved radiolabeling the nanocarrier itself as well as the drug. Either ^{111}In nanoparticle labeling or encapsulated tritiated [^{3}H]paclitaxel revealed that the pH-sensitive nanoparticles had both less uptake in the reticuloendothelial system and an 8-fold lower total body clearance value compared with aqueous formulation, as well as increased drug concentration in the tumor by 23-fold at 5 hours post-administration (98).

Numerous other methods for radiolabeling liposomes for positron emission tomography are possible and have been assessed (19). The ability to track distribution of drug carriers over time is invaluable; it provides feedback during the design process of drug carriers.

Dynamic Imaging: Quantitative Image Analysis

This section discusses examples where quantitative image analysis of drug and vehicle biodistribution provided an assessment on the vehicle's performance to deliver the hydrophobic drug paclitaxel. Paclitaxel retention in liposomes has been problematic and many formulations and their pharmacokinetics have been studied to increase paclitaxel-liposome association (41, 43, 45, 46, 99-105). A long-circulating vehicle that adequately retains paclitaxel is still an unmet need for drug delivery to tumors. Paclitaxel is well characterized for nuclear medicine applications. Various studies on synthesis and analysis of paclitaxel analogues are discussed, followed by imaging studies.

Fluoro-, bromo-, and iodopaclitaxel and their corresponding radiolabeled analogues [^{18}F]fluoro-, [^{76}Br]-,and [^{124}I]iodopaclitaxel have been synthesized and characterized (92). Each of these radionuclides has suitable properties for PET imaging *in vivo*. After synthesis of fluoro-, bromo-, and iodopaclitaxel, the chemical purity of each compound was determined by HPLC and NMR Spectra. The radioactive analogues were also tested for radiochemical purity and specific activity. The radiochemical purity of the [^{124}I] product was 87.3% and remained constant in ethanol for at least 4 days with a specific activity at the *end of synthesis* (EOS) of 2000 mCi/μmol. The radiochemical purity of the [^{76}Br] compound was greater than 95% and remained constant for over 30 hours, while the specific activity at the *end of bombardment* (EOB) was 557 mCi/μmol. The radiochemical yield of the [^{18}F] compound after HPLC purification was 30.3%, while specific activity ranged from 4582 to 12,250 mCi/μmol at the EOB.

Biodistribution studies of the radioactive paclitaxel formulations in rats were also performed. Male Sprague-Dawley rats were injected intravenously with 200 nmol concentrations of radiolabeled paclitaxel. The control was an injection of the drug vehicle, which was ethanol. The organs were removed after sacrifice, mass and radioactive counts were determined so that radioactive content per gram of tissue could be determined for each of the organs of interest. [^{18}F]-paclitaxel and [^{76}Br]-paclitaxel were shown to have rapid clearance from the blood at 60 minutes and high uptake in the liver and kidney. [^{124}I]-paclitaxel had slightly less clearance from the blood, but again had high uptake in the liver and kidneys. In metabolic studies, it was observed that 80% of the radioactivity in the liver was from the parent compound and the rest from metabolites of the [^{18}F] radioligand. In all three studies it was shown that the parent compound was the major component of radioactivity *in vivo*, but metabolite conversion was not insignificant and must be taken into consideration in future studies. *In vitro,* human metabolic studies showed that 50% of the radioactivity after 4 hours is from the parent compound, with only one metabolite responsible for the remainder of the radioactivity. In rats, three metabolites were found. In both cases the [^{18}F] compound was metabolized more quickly than the others.

The outcome of interest in this study as an application of drug delivery validation is that three radiolabeled paclitaxel molecules were synthesized and the biodistributions of all three radiolabeled compounds have been determined. Metabolites of the parent compounds have been found for both rats and humans. These radiolabeled analogues of paclitaxel provide well-characterized payloads for vehicle design and diagnostic studies.

One imaging study utilized [^{18}F]-fluoropaclitaxel to determine if a P-glycoprotein (Pgp) inhibitor effectively blocks the membrane pump's ability to prevent accumulation of paclitaxel in tumor cells in nonhuman primates (88). Multi-drug resistance is known to cause chemotherapeutic failure, in this instance paclitaxel is quickly pumped out of tumor cells such that a therapeutic concentration of drug is not reached (4, 88, 106). Region of interest analysis of full body PET images, yielding time-activity-curves (TACs) for various organs, provided insight into the dynamics of paclitaxel biodistribution with and without pre-administration of the Pgp inhibitor. Further studies on [^{18}F]-fluoropaclitaxel in mice compared PET data to the conventionally derived data by direct organ harvesting (107). This study first suggested that noninvasive PET image analysis was comparable to that of conventional harvesting methods and second introduced the analogous unit, harvested standard uptake value (hSUV), for

comparison with the imaging unit, measured standardized uptake (mSUV). The hSUV was correlated more highly with mSUVs than the conventional percent-injected dose per gram (%ID/g).

Finally, a study using quantitative data from dynamic PET images for various paclitaxel drug delivery vehicles, where radiolabeled paclitaxel or radiolabeled lipid analogues were used to assess drug-vehicle pharmacokinetics. Scanning protocols involved a 90-minute static scan in which only the rat torso was in the field of view for quantitative purposes. Images were reconstructed using maximum a posteriori (MAP) and 90-minute cumulative images and image analysis software were used to draw regions of interest (ROIs). To minimize partial volume averaging effects, the regions were drawn conservatively on the interior of each organ border. The image file was then dynamically binned into 30 frames: 8 x 15 sec, 8 x 1 min, 5 x 3 min, 5 x 5 min, and 4 x 10 min to create time activity curves (TACs) for each organ of interest in the field of view. The mean activity of an organ at each time point was represented as percent-injected dose per cubic centimeter (%ID/cc). The outcomes of these studies are illustrated in Figure 3. Sample 90-minute cumulative maximum intensity projections (MIPs) and TACs are shown for each vehicle formulation, where either the drug was radiolabeled ([^{18}F]fluoropaclitaxel) or lipid components of the vehicle were radiolabeled ([^{18}F]dipalmitoylglycerol, [^{18}F]FDP). Sample TACs are shown for blood and liver, while complete studies provide similar data on numerous organs of interest. In this case, paclitaxel did not remain associated with the vehicle formulations.Both images and quantitative measures reveal that the [^{18}F]fluoropaclitaxel biodistribution did not change between various formulations and is distinct from vehicle and [^{18}F]dipalmitoylglycerol distributions. Quantitative dynamic imaging studies provide the necessary feedback for further development and assessment of paclitaxel vehicles.

CONCLUSION

This monograph has provided a survey of nanotechnology in medicine and a few examples of nuclear imaging techniques. Dynamic imaging elegantly quantifies molecular uptake of radiolabeled substances within tumors and organs of interest over time. It is also capable of providing validation for many other active and extracorporeal targeting strategies. Quantitative imaging was a major focus, however radiotherapies using nanotechnology are on the rise including new chelating agents and novel methods for anti-body and peptide targeting of nanoparticles carrying radiotherapeutic payloads.

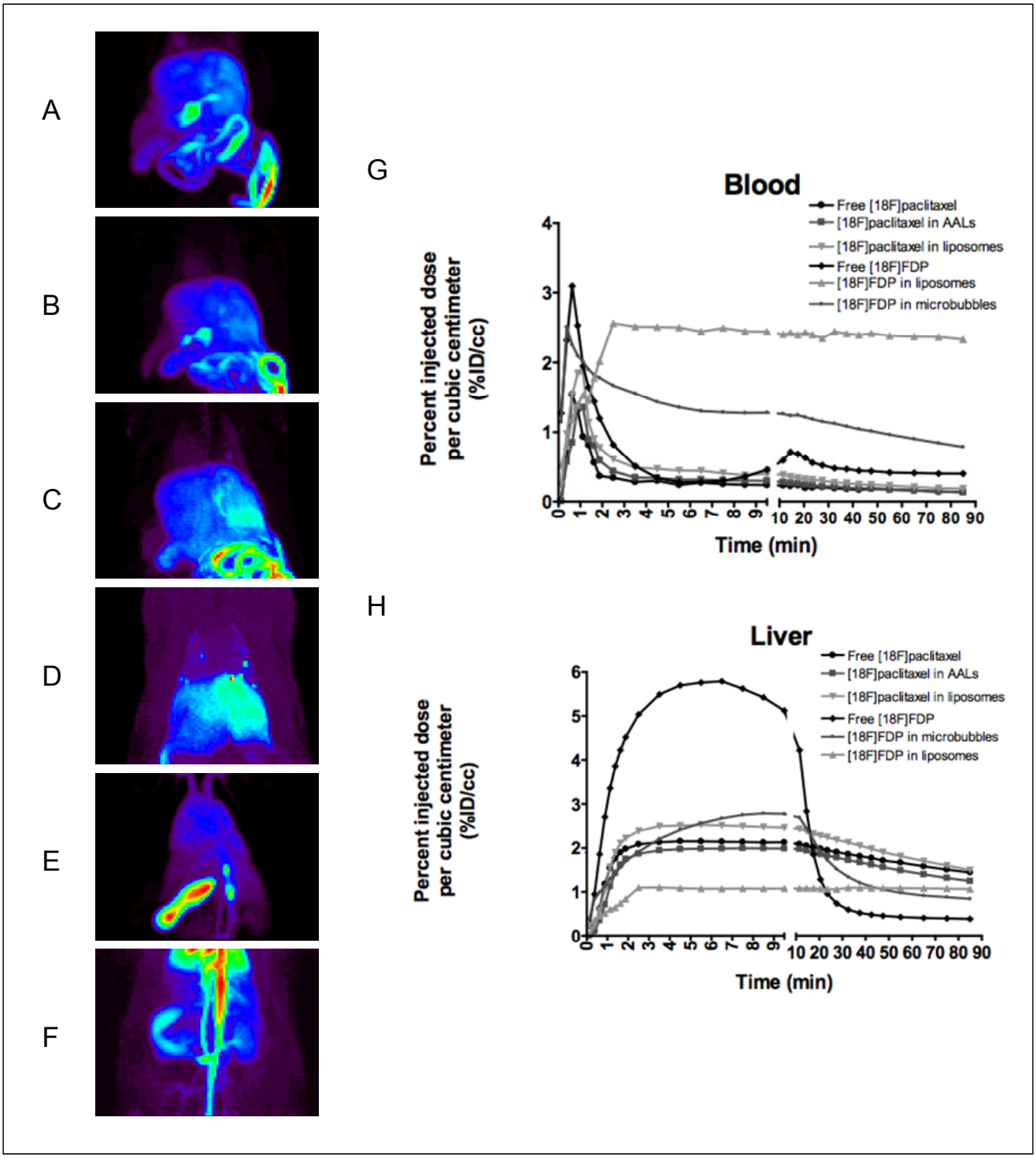

Figure 3. Maximum Intensity Projections (MIPs) of the torso region of rats for various drug and vehicle formulations and Time Activity Curves for Blood and Liver. MIPs of various formulation injections (A) free [^{18}F]fluoropaclitaxel, (B) [^{18}F]fluoropaclitaxel incorporated a hybrid microbubble vehicle called acoustically activated lipospheres (AALs), (C) liposomal [^{18}F]fluoropaclitaxel, (D) free [^{18}F]dipalmitoylglycerol, (E) [^{18}F]dipalmitoylglycerol incorporated into microbubbles and (F) liposomal [^{18}F]dipalmitoylglycerol. Time Activity Curve (TAC) for blood (G) and liver (H), where activity levels are quantified over 90 minutes as percent injected dose per cubic centimeter of blood for each of the six probe formulations

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ASSESSMENT QUESTIONS

1. Nanotechnology can be described as particles with diameters less than_____.

    a. 1.0 micron
    b. 0.1 micron
    c. 100 nanometers
    d. 500 nanometers

2. Which of the following factors within the tumor environment does not contribute to the enhanced permeability and retention effect?

    a. discontinuous basement membrane in tumor blood vessels
    b. increased trans-endothelial transport
    c. inter-endothelial fenestrations
    d. acidic microenvironment

3. While theoretically any molecule can be encapsulated in liposomes, which kind of molecule has the highest loading efficiency?

    a. hydrophobic
    b. hydrophilic
    c. amphipathic
    d. negatively charged

4. Which of the following is not an extracorporeal method to target drug delivery?

    a. ultrasound sonoporation for intracellular uptake
    b. direct injection of compounds to tumor feeder vessels
    c. magnetic ferrofluid accumulation driven by magnetic forces
    d. laser directed thermal ablation

5. Larger particle sizes increase circulation time, however which particle diameter would be capable of avoiding filtration by the spleen?

    a. 100 nm
    b. 300 nm
    c. 500 nm
    d. 1 micron

6. What breakthrough led to dramatically increased lipid based particle circulation time?

    a. polyethylene glycol surface coating
    b. negatively charged surfaces
    c. neutral charged lipid head groups
    d. increased diameters

7. ^{67}Ga-labeling of liposomes has both advantages and disadvantages for gamma imaging, an example of an advantage is its_____.

   a. gamma energy window for image quality purposes
   b. labeling efficiency for image sensitivity
   c. long half life allows longer imaging time points
   d. gamma energy for patient dose

8. Tumors can reach a maximum size of _____ before angiogenesis occurs.

   a. 10 $\mu m^3 \mu m^2$
   b. 200 μm^3
   c. 0.5 mm^3
   d. 1 mm^3

9. Active targeting approaches using antibody and peptide conjugates are hindered by a tumor's_____.

   a. acidic microenvironment
   b. spatial and temporal heterogeneity of receptor expression
   c. blood vessels that are tortuous and disorganized in nature
   d. necrotic core

10. pH sensitive particles release their payload _____ compared with conventional particles at the target tissue site.

   a. unpredictably
   b. more locally at the cell surface
   c. more rapidly
   d. more slowly

11. Hydrophobic drugs are incorporated into liposomes best by _____.

   a. PEG coated liposomes
   b. liposomes containing cholesterol
   c. liposome compositions with the lowest entropic energy
   d. liposome compositions with the highest entropic energy

12. The integrin $\alpha_v\beta_3$ is an ideal target because it _____.

   a. is expressed in all tumor sizes
   b. is expressed in all tumor types
   c. is not expressed in other regions of the body
   d. is over expressed in regions of tumor angiogenesis

13. Laser based approaches for tumor therapy are limited to

   a. tumors undergoing angiogenesis
   b. tumors feeding off neighboring tissue
   c. deep tissue tumors

d. superficial tumors

14. Which vehicles are self-assembling particles with hydrophobic cores?

a. micelles
b. liposomes
c. magneto-liposomes
d. gold nanoshells

15. Microbubbles can be targeted and have had the most success delivering _____.

a. hydrophilic drugs
b. genetic material
c. hydrophobic drug
d. material intracelluarly

16. Liposomes size can be controlled with _____.

a. lipid composition
b. polycarbonate filter extrusion
c. PEG incorporation
d. temperature upon self-assembly

17. PET image analysis cannot _____.

a. provide user-defined region of interest analysis
b. distinguish between parent compound and metabolites
c. dynamically bin of images over time to obtain pharmacokinetics
d. be readily combined with anatomical data

18. Mathematical models that provide relationships between parameters that impact tumor treatment suggest that _____ will improve upon the passive nature of the EPR effect.

a. shorter circulation times
b. receptor and integrin targeting
c. lymphatic drainage and interstitial pressure reduction
d. increased blood vessel density and diameter

19. Which of the following extracorporeal targeting mechanisms could provide treatment to deep tissue tumors?

a. laser photothermal mechanisms
b. electric mechanisms
c. magnetic mechanisms
d. ultrasound mechanisms

20. Incorporating chelators into the lipid bilayer makes direct incubation for radiolabeling liposomes with ^{111}In possible, however _____.

    a. labeling efficiency is low
    b. the resulting particle lacks in vitro stability
    c. the resulting particle lacks in vivo stability
    d. an additional heating step is required

21. Which term refers to strategies combining contrast enhancing capabilities and treatment for cancer?

    a. chemotection
    b. dual-modality
    c. theranostics
    d. nanocarriers

22. The _____ unit describes radiolabeled compound uptake from harvest organs specifically.

    a. hSUV
    b. %ID/g
    c. %ID/cc
    d. mSUV

23. Sonoporation is the _____ permeability enhancement of cell membranes for drug delivery applications.

    a. temporary
    b. irreversible
    c. un-localized
    d. laser-induced

24. For successful delivery of a contrast agent or therapeutic molecule with a nanocarrier, _____.

    a. the molecule must be irreversibly bound to the nanocarrier.
    b. the nanocarrier must be cleared from the bloodstream rapidly
    c. the nanocarrier must be able to take advantage of the EPR effect
    d. the nanocarrier must retain the molecule until intended release

25. Which dual imaging technique provides complimentary functional and anatomical data within the same instrument?

    a. SPECT/PET
    b. PET/Ultrasound
    c. PET/CT
    d. MRI/Ultrasound