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Vascular targeting of nanoparticles for molecular imaging of diseased endothelium

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Abstract

This review seeks to highlight the enormous potential of targeted nanoparticles for molecular imaging applications. Being the closest point-of-contact, circulating nanoparticles can gain direct access to targetable molecular markers of disease that appear on the endothelium. Further, nanoparticles are ideally suitable to vascular targeting due to geometrically enhanced multivalent attachment on the vascular target. This natural synergy between nanoparticles, vascular targeting and molecular imaging can provide new avenues for diagnosis and prognosis of disease with quantitative precision. In addition to the obvious applications of targeting molecular signatures of vascular diseases (*e.g.*, atherosclerosis), deep-tissue diseases often manifest themselves by continuously altering and remodeling their neighboring blood vessels (*e.g.*, cancer). Thus, the remodeled endothelium provides a wide range of targets for nanoparticles and molecular imaging. To demonstrate the potential of molecular imaging, we present a variety of nanoparticles designed for molecular imaging of cancer or atherosclerosis using different imaging modalities.

Graphical abstract

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Keywords

vascular targeting; atherosclerosis; cancer; metastasis; endothelium; MRI; PET

1. Introduction

Historically, nanoparticles have been engineered predominantly for applications that require them to target tissues beyond the vascular endothelium. In this review, we refer to this strategy as ‘deep-tissue targeting’. This comes with no surprise considering that the first success story of nanoparticles was based on exploiting the intratumoral accumulation of particles *via* passive means through the leaky vasculature of tumors due to the enhanced permeability and retention (EPR) effect [1–13]. However, an increasing number of publications indicate that the impact of the EPR effect in deep-tissue targeting is inconsistent, resulting in a patchy, near-perivascular accumulation of nanoparticles in tumors [14–16]. To further increase specificity, targeting ligands have also been employed to direct nanoparticles to upregulated receptors on cells at sites of disease. Precise targeting of cells in diseased tissue (deep-tissue targeting) requires that the nanoparticle has to successfully overcome a series of biobarriers including evasion of the immune system (intravascular transport), extravasation across the endothelium (transvascular transport), navigation through the extracellular space (interstitial transport) and finally meaningful interactions with the intended cell-surface receptors on the target cells. Thus, it is nearly impossible to identify a single design for a nanoparticle that takes under consideration each and every biobarrier. This typically leads to nanoparticles capable of successfully tackling only a subset of the biobarriers, compromising the overall effectiveness of targeting.

Here, we review the rationale and applications of targeting nanoparticles to the endothelium for molecular imaging of biomarkers associated with disease. Besides blood components and plasma proteins, the vascular endothelium is the closest point-of-contact for circulating nanoparticles. By having direct access to the vascular bed, one can envision that nanoparticles can continuously scavenge the endothelium for biomarkers of disease. Thus, considering elimination of two challenging biobarriers (*i.e.*, transvascular and interstitial transport), vascular targeting may be more effective than deep-tissue targeting in many occasions.

While conventional agents of small molecular weight are rapidly distributed within diseased and healthy tissues in a non-specific manner due to the enhanced diffusion of small molecules, we suggest that nanoparticles are highly suitable for vascular targeting due to enhanced targeting avidity as a result of geometrically enhanced multivalent attachment to the vascular target. In fact, the size and the multivalent avidity, due to formation of multiple receptor-ligand bonds, make nanoparticles ideal for targeting of vascular-associated pathologies. However, even if the targeting scheme appears to be simplified than deep-tissue targeting scenarios, one still has to consider the enormous availability of different nanoparticle designs in terms of size, shape and composition, which directly govern the particle’s ability to target a specific vascular site and generate detectable signals using various imaging modalities. Further, depending on the exact diagnostic requirements of a

disease, the design of the nanoparticle has to be mindful of the clinical deployment and radiological imaging. For example, a clinical problem may significantly benefit from the high sensitivity of positron emission tomography (PET) compared to the higher resolution or soft tissue information provided by magnetic resonance imaging (MRI) or x-ray computed tomography (CT). While these considerations may appear overwhelming at first, one of the principle features of nanotechnology is its engineerable nature, which provides multiple degrees of freedom facilitating the fabrication of nanoparticles with the distinct properties required by a specific application.

This review also seeks to illustrate that the objective of molecular imaging of diseased endothelium using nanoparticles is not just to take ‘pretty pictures’ but rather provide new and useful information to physicians and impact their decision-making process. To demonstrate the potential of molecular imaging, we will present applications on two representative diseases, cancer and atherosclerosis. Obviously there are applications of molecular imaging and targeted nanoparticles on many diseases, we selected cancer and atherosclerosis because the field has significantly focused on them. We will first briefly introduce the pathobiology of the two diseases and their characteristics that are related to vascular targeting and molecular imaging (*e.g.*, targetable upregulated biomarkers). For more details about the pathogenesis and development of the two diseases, we guide the reader to comprehensive reviews. In the proceeding sections, we will then discuss design criteria of nanoparticles that dictate their vascular targeting effectiveness. After this discussion, we will review a variety of nanoparticles designed for molecular imaging of cancer or atherosclerosis using nuclear imaging, optical imaging, CT, MRI, ultrasound and multimodal imaging.

2. Disease pathobiology and its influence on the endothelium

2.1 Cancer

Tumor growth often occurs by the successful hijacking of an existing blood supply and the local angiogenesis that follows *de novo* to mediate nourishment of the fast proliferating tumor mass [17]. Largely due to its rapid growth, the tumor microenvironment is strikingly different from healthy tissue [18–22]. The vasculature feeding a tumor is often convoluted and disordered, and blood flow is significantly slowed, transiently stagnant, or even reversed (Fig. 1) [22–24]. There are also a large number of vascular shunts that shuttle blood directly from an arteriole to a venule. Early studies showed that the tumor vasculature is inherently ‘leaky’, where the tight cell-cell junctions between endothelial cells that normally serve to maintain tissue integrity are compromised. In a developing tumor, endothelial cells of angiogenic vessels are often further apart (up to 400–600 nm between cells) [25]. This finding in the early 1980’s, termed the enhanced permeability and retention (EPR) effect, jump-started a burgeoning field where a large spectrum of untargeted imaging and therapeutic agents were delivered *via* the circulation in the hopes that a significant proportion of the dose would be passively retained by the tumor mass [1,26–31]. Certainly there has been some success with EPR delivery of nanoparticles for imaging and therapy. However, this strategy also has serious shortcomings, namely achieving tumor-specific targeting within short timescales, achieving efficient tumor penetration necessary for

therapeutic efficacy, and targeting small tumors such as metastases that may not be well vascularized. Further and importantly, there is a high interstitial pressure barrier within a tumor mass that may be difficult for nanoparticles to overcome to penetrate deeper tissue. As such, in the decades following, efforts to drive efficient, active vascular targeting of nano-agents have gained much attention. Compared to achieving the deep-tissue penetration necessary to target the tumor cells themselves, efforts to preferentially target the tumor-associated vascular endothelium are arguably simpler and intuitively more promising in their increased homing efficacy. Besides applications in molecular imaging, from a therapeutic perspective, methods to mechanically or chemically trigger release of drug cargoes from nanoparticles homing to the tumor-associated vascular bed can serve to deliver therapeutic payloads to deeper tissue as necessary, if vascular delivery is not adequate. From a discovery perspective, vascular-targeted nano-platforms have provided much information regarding tumor progression and pathogenesis. Notably, however, the presence of an EPR effect, no matter how small, can also complicate nanoparticle targeting efforts for molecular imaging of the tumor-associated endothelium. Given that the goal of molecular imaging is to glean quantifiable information regarding the tumor vasculature, in cases where the EPR effect is strong, even vascular-targeted nanoparticles may readily traverse the endothelial layer for retention within the tumor mass, thereby giving a ‘false-positive’ reading. To address this shortcoming, many vascular targeting platforms quantitatively compare targeted particle homing to untargeted particle homing. If necessary, targeted microbubbles can be employed since, by virtue of their larger size ($>1\ \mu\text{m}$), they cannot extravasate into the tumor mass [32].

Contrary to small molecules that are cleared relatively quickly from blood circulation, the prolonged blood residence time of PEGylated nanoparticles generates sufficient ‘interfering’ signal in the lumen of blood vessels to prohibit discrimination of signal from blood vessel walls (*i.e.*, target site) with a high degree of confidence. Thus, imaging must be delayed for days after injection of a long-circulating nanoparticle to allow the agent to clear from the bloodstream. In addition, which leads to a new ‘interfering’ signal from EPR-driven deposition of the nanoparticle. To accurately differentiate vascular targeting from signals in the blood pool or deep-tissue, investigators have optimized and identified a low dose of nanoparticles agents that generated significantly detectable signal on the tumor-associated vascular bed and insignificant background “noise” due to the blood circulation of the nanoparticle or its non-specific uptake in various organs (*e.g.*, liver, spleen) [85,88,87].

In addition to the ‘leakiness’ of the tumor vasculature, there are a host of other microenvironmental factors that drives tumor-specific endothelial cell changes that can be readily exploited for designing targeted nanoparticles. The tumor niche, particularly the necrotic center farthest away from the blood supply, is extremely hypoxic and cells switch to anaerobic glycolysis, resulting in lactate build-up and local acidity. Further, rapid metabolic demands leave the tumor tissue often glucose-/nutrient-starved and there are high levels of oxidative stress on the tumor and associated cells. There is also considerable lymphogenesis where the tumor develops its own lymphatic supply that funnels in tumor-promoting immune cells that drive proliferation and eventual metastasis. Collectively, these processes have a fundamental impact on the tumor-associated endothelium, which divides rapidly and

plays a central role in angiogenesis, in marked contrast to healthy endothelial tissue that is often described as quiescent [18,20,21].

Quite conveniently, endothelial cells are also uniquely transcriptionally rich compared to many other cell types. In response to aberrant tumor-induced environmental stimuli, vascular endothelial cells express strikingly different surface markers, often referred to as a tumor-specific ‘molecular signature’, the complete elucidation of which will prove invaluable for understanding and treating cancer [18,20]. Many high-throughput strategies have been employed to isolate unique tumor-associated endothelial surface biomarkers to exploit these molecules as vascular targets. Microarray analysis, serial analysis of gene expression (SAGE) analysis, and *in vivo* phage-displayed peptide libraries are all methods that have provided key insight. Taken together, key families of endothelial surface molecules have been isolated, many of which are involved in tumor cell migration and metastasis. These include classes of integrins (specifically, $\alpha_v\beta_3$ and $\alpha_v\beta_5$), the family of tumor endothelial markers (TEMs, TEM8 especially), cell adhesion molecules (CAMs), vascular/epidermal growth factors and their receptors (V/EGF and V/EGFR), selectins, cell surface nucleolin, and fibrin-fibronectin complexes [18,20,33]. Markers such as VEGF have been exploited as therapeutic anti-angiogenesis targets in the clinic, with the antibody bevacizumab being a key example [20,34,35].

In addition to relying solely on the ‘molecular signature’ of the tumor-associated vascular endothelium, nanoparticle targeting strategies may benefit greatly from co-delivery of vascular mediators that have been well-established as powerful effectors of the diseased endothelium. For example, co-delivery of potent vasodilators such as bradykinin, angiotensin converting enzyme inhibitors (ACE inhibitors), nitric oxide, prostacyclins, tumor necrosis factor α (TNF α), and nitroglycerin may enhance tumor-specific delivery of targeted nanoparticles [3,4,6,8,9,28,31, 36–38]. This strategy has significant relevance in both diagnostic/imaging and therapeutic applications.

Currently, the choice of clinical imaging modality varies by tumor type and stage of disease. For example, CT and MRI are used widely in scenarios where tumor structure is readily discernible as in the cases of brain or liver cancer, while radionuclide-based methods that can detect tumor cell function with high sensitivity are used to detect smaller tumors as in the case of lymphomas. Across the spectrum of modalities, however, current imaging methods have limitations such as inadequate soft tissue contrast, sensitivity, and resolution particularly at the critical early stage of tumor development when accurate diagnoses and immediate treatment can have tremendous value. As a result, there is a clear need for the development of effective imaging agents, and targeting the tumor vasculature using nanoparticle technology has high relevance in this setting.

2.2 Cardiovascular disease

We focus here specifically on atherosclerosis since it is perhaps the most predominant cardiovascular condition and has benefitted greatly from vascular imaging. As such, much effort has been placed on driving the field forward with the development of nanoparticles as molecular imaging agents of the atherosclerotic endothelium, for both diagnostic and therapeutic purposes. Aberrant changes in cholesterol transport are central for disease

pathogenesis. Cholesterol is moved throughout the body *via* two types of lipoprotein carriers, high-density lipoproteins (HDLs) and low-density lipoproteins (LDLs), commonly referred to as ‘good’ and ‘bad’ cholesterol, respectively. High levels of HDLs are inversely related to cardiac events, as their main functionality is to remove cholesterol from the bloodstream and shuttle it to the liver for excretion. LDLs, the main transporters of cholesterol, are responsible for transporting cholesterol to cells for metabolic functions. Unlike high HDL levels, high levels of LDLs are directly responsible for plaque buildup in the arterial intima, resulting in increased risk of stroke and heart failure. At this stage, this condition is called atherosclerosis [39].

Certain portions of the vasculature are more susceptible to the morphological changes that enable plaque formation. These changes include increased adhesiveness and permeability, and typically occur at vascular sites where blood flow is disturbed, such as at arterial branches, bifurcations, and areas with high vascular curvature. Changes in flow at these sites cause decreased shear stress and increased turbulence, which alters gene expression in the associated endothelium [40]. In addition to flow disturbances, other factors such as smoking, diabetes, hypercholesterolemia, hypertension, and infection all have effects in inducing endothelial cell changes [41]. Endothelial dysfunction is the first step in atherogenesis. The diseased endothelium’s permeability makes it highly susceptible to LDL influxes. High plasma concentrations of LDLs can lead to lumen penetration and accumulation in the intima. Here, LDLs are progressively oxidized to become toxic modified-LDLs; the increased toxicity in the arterial lumen consequently promotes an inflammatory response. Monocytes and lymphocytes emigrate from the blood into the intima, drawn in by the oxidized LDLs and their by-products.

Just prior to this inflammatory immune cell transmigration, endothelial cells upregulate expression of P- and E-selectins, which are involved in the rolling and tethering of monocytes onto the vascular wall. Further endothelial upregulation of molecules of the CAM family (i.e., ICAMs and VCAM-1) increase the adhesiveness of the endothelium, promoting the arrest of the inflammatory cells on the vascular surface. Upon docking, upregulated PECAM-1 assists the monocytes in migrating from the vessel wall to the intima in a process called transmigration [42]. Notably, in contrast to other members of the CAM family, PECAM-1 (CD31) is found at junctions between cells in both healthy and diseased tissue [42,43]. As such, even though it may not serve adequately as a lone target, because of its high expression, it may prove highly effective as a homing agent on dual- or multi-targeted nanoparticles that also display other targets. Integrins are also a key component in the arrest and transmigration of monocytes to the diseased tissue.

Once embedded in the intima, antigen-presenting monocyte-derived macrophages are responsible for scavenging and engulfing the modified-LDLs. In order to continue macrophage recruitment and survival, the macrophages already in the intima will secrete cytokines such as macrophage colony-stimulating factor. Upon continual scavenging, the lipid-laden macrophages develop into foam cells that eventually undergo necrosis after lipid saturation [44]. The necrotic cells eventually release their contents into the lesion site as they die and form a necrotic core, otherwise known as plaque. As a whole, this inflammatory response is chronic since plaques will continue to build in the arterial wall as long as there is

persistence of modified-LDLs in the intima. As the plaque enlarges, the smooth muscle surrounding the artery compensates for plaque growth by expanding itself outward without affecting the lumen lining; this is known as ‘remodeling’ [40]. Early atherosclerosis is non-occlusive as this characteristic is due to arterial remodeling.

Critically, atherosclerosis is difficult to detect using conventional imaging, such as angiography, due to the lack of vessel abnormalities at this stage. As the disease progresses, the atherosclerotic lesion will protrude into the vessel leading to thrombosis. Angiography during this stage will be able to detect lesions, as there is clear vessel deformation that is easily discernible. As the necrotic core continues to expand into the lumen, a fibrous cap overlying the necrotic core will start to thin, making the plaque susceptible to rupture, which may lead to stroke and/or myocardial infarction. Fibrous cap thinning is also caused by flow disruption in the vasculature [45]. Notably, as also discussed previously with respect to targeting tumors, co-delivery of targeted nanoparticles with potent vasodilators such as nitroglycerin may reduce local blood pressure and reverse turbulent flow patterns and may, as a result, have an effect in combatting and treatment plaque formation [8]. Notably, the smaller vessels that make up the vasa vasorum are more susceptible to plaque formation compared to larger vessels, likely because they undergo neovascularization as part of their function in feeding the larger arteries and veins [46]. While not fully established, the endothelial ‘leakiness’ that can result as a part of this vessel remodeling may aid in the collection of macrophages and inflammation mediators that drive atherosclerotic plaque formation [46,47].

Currently, angiography and small molecule imaging modalities are key techniques used in clinical settings for atherosclerosis imaging [48]. Fluorodeoxyglucose (FDG)-PET imaging is also used to visualize and measure macrophage activity [49,50]. While these techniques are efficient and effective in detecting late-stage atherosclerosis, they are unsuccessful in imaging early-stage atherosclerosis or identifying late-stage vulnerable plaques before they rupture [51]. As such, nanoparticle-based imaging of atherosclerotic lesions has been of increasing interest in recent years since molecular imaging will be of tremendous value to effective interventions. To this end, some efforts have focused on macrophage imaging, but because rodent and rabbit models have significantly higher macrophage counts than humans, *in vivo* studies have often not been accurate at correctly depicting human disease. Here, we focus on vascular molecular targeting and imaging of the atherosclerotic endothelium *via* over-expressed endothelial cell markers using prevalent imaging modalities [52,53]. Given that the process of atherosclerotic plaque formation is similar between humans and current animal models with respect to changes in the vascular endothelium, to our knowledge to date, there are no significant discrepancies in clinical translation of results from animal models of the disease.

3. Considerations of blood flow

3.1 Characteristics of healthy vasculature

Blood flow and changes in flow patterns are key contributing factors in the development of cancer and atherosclerosis [54]. Understanding blood flow is also centrally important to the design and engineering of an optimally targeted vascular nanoparticle. The human vascular

system has three primary types of blood vessels: arteries, for distribution of oxygen and metabolites; veins, for collection of metabolic waste and carbon dioxide; and capillaries, the smallest of all vessels, which are exchange points for blood nutrients and tissue metabolites. Vascular endothelial cells, which line all of the vessels in the circulatory system, are at the interface between the blood and the vessel wall. They have a number of functions, including filtration and transport, hemostasis, and neutrophil recruitment. They also play a role in vessel regulation; mechanoreceptors enable sensing of shear stress due to blood flow, which allows vessels to adapt to flow by changing diameter and wall thickness. Blood flow within a vessel is directly proportional to pressure gradients and inversely proportional to vascular resistance. Resistance, in turn, is a function of friction between the endothelial cell lining and the flowing blood. It is inversely proportional to vessel radius to the fourth power such that the larger the vessel, the smaller the resistance, and the faster the flow. Notably, small changes in vessel radius as due to a growing tumor or atherosclerotic plaque can lead to sizeable changes in vascular resistance. Further, blood flow is known to streamline when moving through long smooth vessels. Continuous flow in this manner becomes laminar and is characterized by a parabolic flow profile. Due to their extremely high cross sectional area, capillaries such as those in the center of tumor masses have very slow blood velocities, which enables high interaction of the blood content (i.e., carrying nanoparticles) with the vascular endothelium [54].

3.2 Flow changes during tumor angiogenesis

As a tumor develops and undergoes an ‘angiogenic switch’, an otherwise controlled system of vessels and flow patterns becomes distinctly chaotic [17,21,55,56]. Tumor vasculature is highly dependent on tumor type (including differences between primary tumors and metastases), location, and growth rate. Tumor blood vessel dimensions are different from the host vasculature. They are highly irregular and notoriously leaky, with open endothelial junctions, some perforated endothelium, and often either an abnormally thick or conversely a very thin basement membrane. In dramatic contrast to healthy vessels, blood flow within tumors does not necessarily follow the laminar flow pattern, and there is no dependence on vessel diameter. Flow rates may be orders of magnitude lower than in healthy vasculature, and in some cases may even reverse direction. The variability in flow rates may be due in part to the increased resistance in tumor blood vessels. Another factor that can play a role is the opposing forces that govern the high permeability of the tumor vasculature versus the interstitial fluid pressure. Collectively as a result of these vascular flow changes, tumor vessels are often susceptible to blood clot formation [5,57,58]. Finally, rapidly proliferating cancer cells generate a solid stress on tissue, which may contribute to flow effects (Fig. 2) [59].

Blood flow rates are heterogeneous throughout tumor tissue, which in general is divided into regions based on vascularization and permeability [21,60]. The central-most regions are avascular and necrotic, with low flow rates, while the surrounding regions contain a stabilized microcirculatory system that typically has highly variable flow rates. In many cases, increases in systemic blood pressure has been shown to increase blood flow to tumors in comparison to healthy organs, but these flow increases are notably not homogenous throughout the entire tumor mass [5,7,61]. This heterogeneity can complicate efforts to

disperse therapeutic agents throughout the entire tumor mass. While it may be possible to deliver agents to the well vascularized, highly permeable region at the periphery of a tumor, these molecules have to rely on diffusion to reach the center. This diffusion process is slow, and in addition, these molecules may be carried away from the central regions by convection of interstitial fluid. This physiological makeup is a key driving force for developing targeted nanoparticles that home to the tumor vascular face *in lieu* of particles that must penetrate deeper into the tumor tissue [2].

3.3 Flow changes during atherosclerotic plaque development

Blood vessels are subject to a number of hemodynamic forces. Cumulative changes in these forces play a central role in atherosclerotic plaque formation. Endothelial cells in particular are sensitive to flow patterns and fluid shear stress. In straight vessels where flow is laminar, wall shear stress is high and directed along the vessel surface. Changes in shear stress play a critical role in vascular homeostasis [62]. When blood flow transitions from laminar, parabolic flow to turbulent flow, blood flows across the vessel (and no longer along it) and eddy currents are formed. Eddy currents significantly increase the resistance to blood flow. Turbulent flow is a direct function of blood velocity and an inverse function of viscosity. Turbulent flow also occurs when there are changes in the structure of a vessel, such as a sharp turn, a rough surface, or an obstruction (Fig. 3).

There are a number of conditions that lead to disturbed flow in the vascular system. These include natural disturbances due to branch points and curved arteries, and disturbances created through surgical interventions, such as stent placement. While some conditions are lasting, others, such as flow changes during organ transplantation, may be temporary. Disturbances in flow contribute to a number of clinical disorders, including atherosclerosis and arterial aneurysms [63]. Disturbed flow also results in lower shear stress and creation of shear stress gradients and is known to upregulate endothelial cell genes and proteins that promote atherogenesis. For example, shear stress-induced production of endothelial nitric oxide synthase (eNOS) in the vascular endothelium also serves to increase local blood flow, which in turn can exacerbate the formation of eddy currents and plaque development [36,37]. As a result, disturbed flow can lead to localization of atherosclerotic lesions. The growing plaque may develop a positive feedback loop, continuing to disrupt blood flow to feed its growth, which may potentially lead to a vessel obstruction. Interestingly, the rate of atherosclerosis progression can vary even between segments of the same vessel, because it is dependent on highly local differences in flow disturbance. In advanced lesions, weakened artery walls can lead to pressure-induced aneurysms [62,63].

4. Nanoparticle design

4.1 Nanoparticles versus small molecules, antibodies, and aptamers

Design considerations for optimal vascular targeting agents involve evaluation of the properties of several major classes of molecules [18,26]. Small organic molecules can be synthesized and functionalized easily, penetrate rapidly and deeply within tissues, and can be structurally altered to control their pharmacokinetics. The chief disadvantage here, however, is achieving high-affinity binding, which has serious implications in targeting and

imaging accuracy. In comparison, monoclonal antibodies have perhaps the highest target binding affinities (micromolar to picomolar dissociation constants) among ligand classes. They can be raised to bind to nearly every target and, when applications require smaller ligand sizes, antibodies can be decoupled into smaller component fragments that retain similar functional binding capability. Peptide ligands, often only ~3–12 amino acids in length, are advantageous because of their relatively small size (even smaller than antibody fragments). They typically have dissociation constants in the micromolar range, but further improvements in affinity can rarely be achieved because of limited high-affinity binding peptide sequences available for some antigens. Aptamers can be easily prepared with dissociation constants in the micromolar to nanomolar range. The challenge here, however, is the necessity for thorough testing to ascertain properties that govern stability *in vivo* [18].

Compared to these classes of molecules, the use of nanoparticles significantly broadens the scope for ‘engineerability’ of unique agents tailored specifically for imaging of the diseased endothelium [20,21,60,64]. Nanoparticle technology spans a vast gamut of potential material choices (or combinations of materials). These include polymeric materials that can be constructed to be biodegradable, metallic particles, lipid-based particles or liposomes, nucleic acid-based particles, and protein-rich materials, all of which have unique benefits and drawbacks that can be tuned in an application-dependent manner [65–68]. Methods of chemistry can also be adapted to synthesize particles of various sizes and shapes, including the more traditional spheres and rods as well as nano-flowers, -cubes, -worms, -chains, -discs, -stars, and even nano-snowflakes [66,69]. This tuning of nanoparticle structure can play a critical role in targeting efficiency, which is important in vascular targeting applications where particles have to maneuver through the blood circulation. Furthermore and very importantly, particles can often be functionalized with targeting moieties and agents that confer important biological properties such as water-solubility, endowed with imaging capabilities that may not be intrinsic, and, most often, encapsulated with drug payloads that must be delivered for therapeutic efficacy. Notably, while the FDA has approved some relatively inert nanoparticle formulations such as certain liposomes for use in clinical trials, approval for the majority of particles that are currently at the pre-clinical stage will require comprehensive toxicity analysis for both short- and long-term side effects [70].

4.2 Nanoparticle size, shape, and surface composition as central design criteria

Vascular targeting requires that particles are able to tumble out of the general blood circulation, marginate along vessel walls, and attach with relatively high affinity to their cognate targets. The endothelial landscape and the flow and shear considerations involved can vary significantly. For example, particles that are built to target a well-vascularized primary tumor may benefit from the sluggish blood flow within the irregular tumor capillaries and accurate targeting of the entire tumor vasculature may not be much of a challenge. By contrast, particles constructed to target metastases or even early micrometastases that grow intimately along the endothelium of larger vessels, have to overcome considerably larger flow velocities and shear stresses in order to home successfully. Size, shape, and surface composition are key governing factors that define efficient particle delivery and function. These criteria vary by nanoparticle application and here, we discuss considerations specifically important for the design of particles for vascular

targeting in terms of their influences on particle pharmacokinetics, margination, and avidity of binding.

Particle size plays a central role in their clearance, which has important implications for toxicity related to long-term retention. Particles less than 10 nm in size are cleared rapidly *via* the renal filtration system, while larger particles are cleared by the liver and spleen [71–74]. Further, it has been shown that targeting of nanoparticles in different microenvironments varies dramatically (anywhere from one to three orders of magnitude) depending on the particle size. In general, smaller particles are able to marginate along vessel walls more avidly than larger particles that tend to move along with the general circulation [69]. Specifically in the tumor-associated vasculature, particle size appears to play a role in targeting efficiency. FMT imaging studies using vascular EGFR-targeted liposomes of smaller (30 nm) and larger (100 nm) sizes in mice with primary breast tumors showed that targeting of 30-nm liposomes was significant compared to untargeted 30-nm liposomes, untargeted 100-nm liposomes, and even targeted 100-nm liposomes when blood flow rate is slow as in the core of the tumor mass [61]. However, when flow rate increases, there appeared to be no difference in deposition between targeted and untargeted liposomes, but 100-nm liposomes accumulated much more significantly at the tumor and associated vasculature than 30-nm liposomes. In regards to binding avidity, larger particles enable more multivalent decoration with targeting moieties, and, as such, favor greater binding compared to smaller particles [75–77].

Compared to spherical particles, asymmetric oblong particles such as nanorods and discs appear to have important shape-based advantages with respect to circulation and binding avidity (Fig. 4). While spherical particles can be cleared by macrophages of the mononuclear phagocytic system (MPS, also known as the reticuloendothelial system) of the liver and spleen, rod-shaped particles are endocytosed to a lesser degree [78–80]. Because of lower clearance rates, rod-like particles may circulate longer. Further, because oblong particles are subjected to torque forces within blood flow, they have a tendency to tumble out of the general circulation and ‘scavenge’ along vessel walls [81–85]. This concept has been uniquely illustrated by studies using iron-oxide nanochains, which are constructed from 3–4 nanospheres covalently linked together [85–91]. Nanochains were targeted to vascular endothelial and tumor cell-associated markers and, in studies involving imaging and treatment of tumors that are notoriously difficult to target such as breast tumor micrometastases and invasive brain tumors, nanochains were able to marginate to tumor sites within only 1–3 h of injection significantly more than individual iron oxide spheres or liposomes. Finally, in terms of binding avidity, oblong particles may offer greater multivalency than their spherical counterparts, but they can also offer higher avidity since ligands are arranged on a planar surface and available in greater numbers in comparison to spherical particles where curvature limits the number of ligands available for binding at a given instance [92,93].

Nanoparticle surface chemistry dictates the first point-of-contact a particle has with its environment and governs the efficiency with which it binds to its cognate target receptor. Vascular targeting requires that particles are soluble in the blood and can remain in circulation for at least several hours post-injection [94]. To this end, particles are often

coated with water-soluble polymers such as polyethylene glycol (PEG). For accurate targeting, particles must be functionalized with a critical mass number of surface ligands [94–96]. Several studies have explored targeting effects with more than one type of ligands under the premise of achieving higher deposition at target sites [96,97]. This strategy of ‘multi-targeting’ is especially relevant when nanoparticles exploit endothelial surface markers that vary by disease stage. Using more than one target offers a greater chance of targeting a tumor growing between stages that can otherwise escape detection and improves overall targeting accuracy of the tumor as a whole.

4.3 Targeting strategies

So far, we have discussed how the design of a nanoparticle dictates its biochemical and biophysical interactions with the target site. First, the biophysical interactions of nanoparticles with the vascular bed can be tweaked by changing their size and shape. Second, due to their size, nanoparticles can be decorated with a large number of ligands, which significantly augments the biochemical interactions with the complex tumor microenvironment. Furthermore, presentation of ligands (*e.g.*, length of PEG) and ligand density can be controlled on the nanoparticle’s surface. The number of nanoparticles targeting a cell-surface receptor is maximized at an optimal number of ligands per particle. Once this point is crossed, the ligands in excess unnecessarily consume receptors resulting in early receptor saturation and diminishing the targeting likelihood of forthcoming particles.

In particular, the vascular bed upregulates specific biomarkers that often reflects the state of the diseased tissue behind the endothelium. To increase targeting accuracy, various ligands and targeting strategies have been used to image vascular biomarkers of disease that are different from healthy tissues. Obviously, the selected targets should be minimally expressed on normal resting blood vessels. As discussed in section 2.2, in the case of atherosclerosis, typical targets include the CAM family (*i.e.*, ICAMs and VCAM-1), integrins, selectins and other adhesive molecules involved in the recruitment of leukocytes in inflammation. Notably, a subset of these biomarkers are also targetable in cancerous tissues, since they are key players in the process of angiogenesis and metastasis (section 2.1). Importantly, the early spread of metastasis to distant organs lacks the typical angiogenic activity seen in many types of solid tumors. Even though typical angiogenic markers are not available for targeting in the case of metastasis, many of the adhesive molecules found in inflammation are present (*e.g.*, integrins, selectins). Considering that the early metastatic spread of cancer cells involves extravascular metastases (*i.e.*, cancer cells residing inside the lumen of blood vessels), the cancer cells carry a continuously evolving phenotype including varying overexpression of cell surface receptors (*e.g.*, folate, EGF, HER2, CD30, integrin receptors).

Another important consideration is that atherosclerosis and cancer should not be approached as monoliths. They are both highly dynamic diseases with cells evolving and changing over time and space, including the expression of targetable cell-surface receptors. Thus, it is important to capture this dynamic microenvironment, which is missed by a traditional single-ligand strategy. This is another major advantage of nanoparticles over single molecule targeting probes. Presentation of different types of ligands can be easily controlled on the surface of nanoparticles in terms of ligand density and ratio among different ligand types.

Such multi-targeting system is ‘adaptable’ to the dynamic nature of disease by considering the spatiotemporal changes of targetable receptors within the same patient, leading to effective targeting of different locations or regions of the disease that predominantly express different targetable receptors at any given time.

In the next section, we review different nanoparticle platforms and their applications on molecular imaging of cancer or atherosclerosis using different imaging modalities.

5. Molecular imaging applications

5.1 Particles for nuclear imaging

Nuclear-based methods for imaging such as single-photon emission computed tomography (SPECT) and PET are among the first techniques developed for vascular targeting applications. Since radionuclides can be detected with very high sensitivity (in the pM range) without issues with deep tissue penetration and signal can be easily quantified, nuclear imaging has become the standard modality for molecular imaging in particular. Its chief limitation, however, is its relatively low spatial resolution at ~5 mm. As a result, compared to the number of nanoparticle-based targeting methods that solely rely on nuclear imaging, there are a large number of nanoparticle platforms developed using multimodal imaging (see section 5.6). In these cases, multimodal studies rely on nuclear methods for sensitivity and other modalities for high spatial resolution. In section 5.7, we summarize all the key features of the different imaging modalities and provide an overall perspective on selecting the appropriate nanoparticle for molecular imaging applications in terms of regulatory approvals and clinical adaptation.

In the case of cancer and molecular imaging of angiogenesis in particular, a large number of studies have involved radiolabeled antibodies that bind with high affinity to well-established biomarkers such as VEGF/VEGFR with both SPECT and PET [98]. More specifically, ^{123}I [99,100], ^{125}I [101], and $^{99\text{m}}\text{Tc}$ [42], [102–104] have been used as radiotracers attached to VEGF/VEGFR for SPECT imaging, while ^{124}I [75,76]. Additionally, ^{111}In and ^{89}Zr have been attached to bevacizumab for SPECT and PET studies, respectively [106]. In the particle landscape, ^{64}Cu -labeled, $\alpha_v\beta_3$ integrin-tagged single-walled nanotubes (SWNTs) were also used for angiogenesis imaging *via* SPECT [107]. $^{99\text{m}}\text{Tc}$ -labeled liposomes co-labeled with ligands for both P-selectin and $\alpha_v\beta_3$ integrins were used to effectively corroborate by SPECT imaging short-term early targeting at vascular sites of breast metastasis that was initially imaged by optical imaging (fluorescence molecular tomography or FMT) [97]. Similarly, gold nanoparticles targeted to vascular endothelial/tumor cell $\alpha_v\beta_3$ integrins were co-labeled with $^{99\text{m}}\text{Tc}$ and SPECT imaging was used to quantify the minimal particle dose that reached lung metastases within only 1 h of injection (Fig. 5). Notably here, because of the extraordinary sensitivity of SPECT imaging, measurements could be made using a very small dose of gold nanoparticle [91]. Other studies using gold particles targeted to vascular endothelial/tumor cell $\alpha_v\beta_3$ integrins and co-labeled with ^{125}I showed by SPECT/CT imaging that effective targeting occurred rapidly within 10 min post-injection at brain tumor sites and that particles were endocytosed for longer-term retention [108]. Similarly, PET imaging has also been used for vascular targeting of the tumor-associated endothelium. CD105-targeted gold nanoshells [109] and integrin-targeted mesoporous silica nanoparticles

[110], both co-labeled with ^{64}Cu , have been used in PET studies of the vascular endothelium.

By contrast, advances in nuclear imaging with regards to the use of nanoparticles for vascular targeting of the diseased endothelium in atherosclerosis have been limited. SPECT studies using $^{99\text{m}}\text{Tc}$ -labeled single domain antibody fragments with nanomolar affinities to human and mouse VCAM-1 (cyclic antibody VCAM1-5, cAbVCAM1-5) was used to monitor expression levels following anti-atherogenic treatments. VCAM-1 expression appeared to be directly correlated to plaque size [111].

5.2 Particles for optical imaging

Optical imaging methods, particularly those that are fluorescence-based, are extensively used in pre-clinical studies for their cost-effectiveness, high sensitivity, absence of ionizing radiation and related toxicity, and ability for imaging in real-time. There is, however, a notable difference in achievable spatial resolution between microscopy-based optical methods (e.g., confocal imaging) that can readily discern even sub-cellular structures ~100 nm apart and small animal optical imaging where resolution is limited to a few millimeters [22]. Significantly, this discrepancy has important implications for accurate molecular imaging since resolution is an important parameter. Further, while confocal imaging is combined with multi-photon imaging to achieve deep tissue penetration and to minimize autofluorescence in pre-clinical settings, clinical imaging methods are often limited to the use of near-infrared (NIR) dyes because only these wavelengths can penetrate tissue for depth imaging.

In the case of cancer and molecular studies of the developing tumor-associated endothelium in bone marrow-homing acute lymphoblastic leukemia (ALL) cells, *in vivo* immunofluorescence confocal/multi-photon microscopy using intravenously injected antibodies targeted to overexpressed endothelial selectins and CAMs have been used extensively for dynamic imaging purposes [112]. Because fluorescent probes can be readily attached to antibodies that inherently have very high binding affinities, this method of imaging was developed early and represents a simple and highly sensitive method in studies of vascular targeting of diseased endothelium. In comparison, fluorescent nanoparticles (*i.e.*, liposomes [113], silica [113], polymersomes [113], rare-earth nanocrystals [114]) are used to achieve longer retention at target sites and associated vasculature, for multimodal imaging studies, and to simultaneously carry drugs for delivery. For example, fluorescent cationic liposomes ~50 nm in diameter have shown a significant increase in affinity for angiogenic endothelial cells in pancreatic islet tumors compared to neutral and anionic liposomes as quantified by confocal imaging analysis [115–117]. Engineered fluorescent viruses have also been used for vascular targeting studies. Filamentous bacteriophages constructed to express targeting peptides and used to isolate tumor vascular targets, have been shown by confocal microscopy to bind with high affinity to upregulated $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins in the pancreatic islet tumor vasculature *via* RGD-4C and RGD [118–120]. Inert, biocompatible, and multivalent plant viruses such as the cowpea mosaic virus (CPMV) have also shown to accumulate preferentially in the periphery of the vascular lumen and have been used in confocal and intravital imaging studies of neo-angiogenesis of even the smallest of

capillaries [121]. Further, *in situ* three-dimensional fluorescence cryogenic imaging (3D cryo-imaging [122–124]) that allows for whole-body imaging has also been used for imaging/detection of small metastatic tumors (less than 0.5 mm) and associated stroma/endothelium following intravenous injection of fluorescent peptides that target the upregulated fibrin-fibronectin complexes [125].

As discussed earlier, clinical imaging modalities have largely relied on the use of NIR dyes to achieve deep tissue penetration. Fluorescence molecular tomography (FMT) studies have shown that NIR dye-tagged liposomes functionalized with two endothelial/tumor cell targets (specifically, for P-selectin and $\alpha_v\beta_3$ integrins) have significantly greater targeting efficiency in models of triple-negative breast cancer compared to liposomes functionalized with only a single target [97]. Similar FMT studies with NIR-tagged EGFR-targeted liposomes have shown liposome size-dependent deposition within primary mammary tumors as a function of local blood flow measured in parallel by micro-CT [61]. Quantum dots (QDs) have shown much potential for clinical imaging because of their high signal intensity but their utility for medical imaging/diagnosis has been severely limited by their toxicity [126–133]. Recent efforts to synthesize QDs that are biocompatible, NIR-fluorescent, ultra-small (5-nm), stable *in vivo*, and cleared *via* the renal circulation, have however been promising [134]. Dendron-coated InP/ZnS QDs targeted to $\alpha_v\beta_3$ integrins in both ovarian tumor cells and associated vasculature resulted in high signal retention lasting longer than 24 hr compared to untargeted QDs [134]. Not surprisingly, NIR-tagged RGD-labeled quantum dots (QDs) have been used to visualize tumor-associated vascular $\alpha_v\beta_3$ integrins with intravital imaging [135,136]. Finally, besides fluorescence-based techniques, optical imaging methods that rely on the strong surface plasmon resonance properties of metallic particles such as gold spheres [137–139], rods [140,141], and nanocages [142–144] targeted to tumor-associated vascular EGFR have also been developed.

In the case of vascular targeting of atherosclerosis and *in vivo* optical imaging, NIR dye-tagged magneto-optical particles have been used to target VCAM-1 and monitor expression changes in real-time using intravital imaging [145–147]. Similarly, plaque-associated VCAM-1 has been targeted using ultrasmall SPIO particles and imaged with combined optical, SECARS, and electron microscopy [148].

5.3 Particles for CT

X-ray based techniques such as CT, mammography, and tomosynthesis are among the oldest and most widely used clinical imaging modalities today. CT-based imaging is often the first method of choice for detection of cancer and cardiovascular disease. It is widely available in clinical settings and has key advantages including low cost, quick scan times, very high spatial resolution, and precise signal quantification. As early as in the 80s, lipid-based contrast agents were developed for tumor imaging. For instance, Lipiodol is often used to exclude benign from malignant lesions in the liver and kidneys [149–151]. There is, however, an inherent risk of exposure to harmful ionizing radiation, which is increasingly minimized with newer generation scanners. Still, the chief limitation of CT-based techniques particularly using nanoparticles for molecular imaging is the low-contrast sensitivity of x-rays (in the mM range) that limits soft tissue discernment [64]. As a result, detection

currently relies on increased expression of target biomarkers where contrast agent can accumulate at the high concentrations requisite to overcome the detection threshold. However, this necessity is a significant obstacle in detecting early stages of vascular disease where biomarker expression is often sparse. Iodine has been the most widely used x-ray contrast agent in clinical CT imaging. For instance, iodine has been encapsulated within liposomal nanoparticles and used to characterize the tumor vasculature studies in mice bearing mammary tumors using micro-CT imaging (Fig. 6) [61]. There is, however, much effort invested in developing CT contrast agents that have higher x-ray attenuation and better contrast. High atomic weight metallic nanomaterials such as gold, bismuth, and rare-earth elements have been studied for this purpose. Notably, because many of these metallic particles are non-degradable, toxicity and clearance analysis is an important consideration in developing novel CT nanoparticle agents for clinical translation.

In the case of cancer, gold nanoparticles (both spheres and rods) have been readily functionalized with vascular endothelial (EGFR [152]) and tumor cell (HER2 [150,151], PSMA [155], folate receptor [156], UM-A9 [157]) targets for molecular CT imaging, where studies have shown marked differences in retention in tumor-associated areas compared to untargeted particles. Specifically, EGFR-targeted gold particles enabled effective CT visualization of the tumor-associated vasculature in mouse head and neck carcinoma where tumors are a comparatively small ~4–5 mm [152]. Similarly, bismuth-sulfide particles attached to LyP-1 targeting the tumor-associated lymphatic endothelium and (to a lesser extent) tumor cells themselves allowed CT-based imaging of breast tumors even 24 h after injection [158]. Further, upconversion nanoparticles, which are rare-earth/lanthanide-doped (Gd-, Yb-, Lu-doped) particles, are naturally luminescent and can convert lower energy radiation to higher energy radiation.

In the case of atherosclerosis, coronary CT angiography is used widely for patients with mild to moderate coronary artery disease and mild angina. Limited soft tissue discrimination due to inherently low contrast agent accumulation in atherosclerotic lesions has been one of the chief shortcomings of the field. Efforts to improve CT imaging have started with scanner enhancements to reduce artifacts, noise, and decrease radiation doses. Further, coupling CT angiography with calcium subtraction methods has been shown to improve image quality [51]. Another limitation of this technique is its intrinsic spatial resolution. Lesions can be imaged but little information can be gleaned to accurately describe plaque morphology specifically fibrous cap size. Effective analysis of plaque morphology can be used to predict and isolate vulnerable plaques (before rupture). As such, efforts to address these limitations have focused on the developing of contrast agents with higher x-ray attenuation. Metallic nanoparticles in particular can play a beneficial role.

Gold particles have been exploited in this setting for their high x-ray contrast and because they can be readily functionalized. Gold particles coated with HDLs have been used to mediate particle targeting to atherosclerotic plaques specifically, enabling plaque detection and also macrophage recruitment [159], [160]. Crystalline iodinated particles, while less sensitive than gold particles, have also been used for plaque targeting and analysis of macrophage entry [160]. Notably, these studies have focused on targeting plaques to identify macrophage burden and calcification in vulnerable plaques. For the purposes of imaging

plaque-associated endothelium for diagnostic and therapeutic monitoring purposes, further advances must focus on improving CT spatial resolution, which must be high in order to achieve detailed vascular information.

5.4 Particles for MRI

Compared to other imaging modalities, MRI provides high soft tissue contrast and good spatial resolution [161]. There is a substantial amount of work that has been done in the development of targeted nanomaterials for imaging of the diseased endothelium in both cancer and cardiovascular disease. For cancer, both T1 and T2 agents have been developed. Specifically, in the case of T1 sensitivity, the paramagnetic properties of gadolinium (Gd) have been exploited widely. For example, Gd has been attached to small-molecule peptides and lipid-based nanoparticles (i.e., liposomes and lipid-based emulsions) for imaging of angiogenesis and tumor vasculature-associated matrix [159,160]. Vascular targets include ICAM-1 [164], VCAM-1 [145], CD105 [165], $\alpha_v\beta_3$ integrins [163–165], fibrin-fibronectin [125], and even multiple targets on the same carrier [166,167]. Nanoparticles are particularly advantageous here compared to small-molecule agents because of the increased multivalency they provide for Gd decoration, which in turn provides a significant improvement on relaxivity [171]–[173]. Along these lines, dendrimers are also highly multivalent and ultra-small dendrimers (5–10 nm in size that drives renal clearance) have been used for Gd-based targeting/imaging of folate receptor-expressing tumors and associated vasculature [174]. Gd-based agents have, however, proved toxic when clearance is impaired as in the case of patients with compromised renal function. As such, there are efforts to develop targeted MR agents with alternate nuclei, chiefly manganese, cobalt, and nickel [175]. In efforts to achieve minimal background signal, fluorine- [173,174] and silica-based MR probes [175,176] are being developed and have shown dramatic improvements in sensitivity, resolution, and quantitation.

Similarly, in developing T2-based particles, much effort has focused on the development of superparamagnetic iron oxide (SPIO) nanoparticles chiefly because they have high T2* sensitivity and detection thresholds in the micromolar range and allow for easy functionalization with a variety of targeting ligands [180]. Optimal MR imaging with SPIO particles depends on their magnetic properties, which, as studies have shown, depend on particle size and shape [181], [182]. SPIO particles functionalized with integrin targets [183] and VEGFR [184] have been used for molecular MR imaging of the tumor-associated endothelium. By further engineering of SPIO particles to form a chain, recent studies have shown targeting to tumor vascular integrins resulted in increased detection of breast tumor metastasis using MRI [85]. Given that their chain-like structure display geometrically enhanced multivalent targeting, these studies have shown that nanochains appear to attach to vascular targets with greater efficiency compared to their spherical counterparts. Targeting nanochains to $\alpha_v\beta_3$ integrins upregulated in metastatic foci and the associated vasculature resulted in significant retention compared to spheres even in micrometastases (less than 1 mm in size) that are, as yet, clinically undetectable [86–89].

Alternatively to methods that exploit T1 and T2 contrast, liposome-based chemical exchange saturation transfer (lipo-CEST) MR particles have also been developed [182,183]. Here,

using specific radio frequencies to discern proton signals from various molecules, CEST small-molecule agents encapsulated in liposomes can help to increase contrast and reduce background by several orders of magnitude [187]. Lipo-CEST particles targeted to upregulated $\alpha_v\beta_3$ integrins were shown to home with high efficiency to angiogenic vessels of brain tumors [188].

MR imaging does not require radioisotopes or radiation, making it a more viable and safe modality with vulnerable patients. However, atherosclerosis imaging can be difficult using MR due to its long acquisition times and often sub-par signal-to-noise ratios primarily caused by motion artifacts; this behavior is typically exhibited in the coronary artery making it one of the most challenging arteries to image. Efforts to improve motion artifacts have included black-blood MR, increased magnetic fields, use of contrast agents, and other motion correcting/compensating tools [189]. In order to acquire improved MR contrast-to-noise ratio, research has focused on diseased endothelial vascular targeting and associated plaque accumulation contrast enhancers, which include liposomal Gd [190], SPIO nanoparticles [146,148,188], monocrystalline magnetic nanoparticles [145], and Gd lipid-based emulsions [189,47,190]. Many of these techniques have overlap vascular targeting for cancer applications with uses in diagnostics and therapy.

VCAM-1 targeting has been of great interest in the field. For example, *in lieu* of antibody-mediated targeting, phage-display selection methods were used to identify several VCAM-1 peptide sequences that were amenable for rapid internalization upon contact with endothelial target cells. Further selection criteria included similarity to surface sequences on circulating hematopoietic cells and ability to attach to a fluorescence probe. Attachment of the resulting peptides to monocrystalline iron oxide nanoparticles showed that, in marked contrast to antibody-mediated targeting, there was a significant increase in signal-to-noise ratio at target sites and subsequent endothelial cell uptake and long-lasting accumulation. Optical imaging of the attached fluorescence probe was used to corroborate these MR findings (Fig. 7) [145]. Other multimodal particles have been used for spatial monitoring of VCAM-1 expression in lumen of diseased vessels [194]. Ultra-small SPIONs have also been used to target both VCAM-1 and P03011 [25]. Specifically in the case of P03011-targeted SPIONs, a high magnetic field was used to detect plaque accumulation with increased spatial resolution. However, while possible in preclinical studies, increasing the magnetic field as in this study is an engineering disadvantage when translating this technology for clinical use. Iron oxide microparticles have also been developed for MR imaging of plaques and associated vasculature. Specifically, dual-targeted microparticles decorated with both VCAM-1 and P-selectin antibodies showed a significant targeting advantage compared to particles targeted with individual ligands [195].

Integrins, primarily $\alpha_v\beta_3$ integrins, are another targeting ligand family widely used for plaque and plaque-associated vasculature targeting. Atherosclerosis is known to promote angiogenesis, which aids in the further development of plaque size and later plaque rupture. In particular, $\alpha_v\beta_3$ integrins are known to mediate this angiogenesis. Monitoring the effects of angiogenesis *via* $\alpha_v\beta_3$ integrin imaging can also lead to detection of vulnerable plaques [189,47,190]. Initial MR imaging studies of angiogenesis in atherosclerosis involved the use of Gd-loaded lipid-based emulsion particles targeted to $\alpha_v\beta_3$ integrins [192]. Further, Gd-

loaded HDL particles [196] and SPIONs targeted to macrophages and apoptotic cells [146,188] within the plaque lesion were also used for MR imaging of these sites.

5.5 Particles for ultrasound

US imaging is a low cost modality with the added advantages of high contrast and spatial resolution. Because many US agents have short half-lives, US imaging is particularly suited to targeted vascular imaging since particles dock at their sites often within the first several hours of injection. Notably, significant focus has been placed on the development of submicron- and micron-sized carriers for the purposes of US imaging. In the case of cancer, over-expressed vascular VEGFR-2 [35,194] and $\alpha_v\beta_3$ integrins [195–199] have been targets of choice for bubbles using both peptides and antibodies as targeting moieties. In particular, bubbles targeting VEGFR-2 have been used to monitor this receptor expression during treatment inhibiting angiogenesis [35,195]. Further, micro-bubbles functionalized with both VEGFR-2 and $\alpha_v\beta_3$ targets have also shown significantly higher homing potential compared to bubbles with only one targeting species [203].

Contrast-enhanced US is of increasing interest in atherosclerosis imaging due to low cost, widespread availability, and short imaging time. Bubbles are gas-filled lipid bilayers that undergo volumetric oscillations, size shrinkage and expansion, under an acoustic field, promoting significant contrast-enhancement in US. VCAM-1-targeted microbubbles have been used to measure vascular inflammation in lesion sites [201,202]. Quantification of the expression levels of VCAM-1 at different stages of atherosclerosis can lead to valuable information that could enable classification of disease stage and associated biomarker expression. With the use of low frequency and high amplitude ultrasound waves, bubbles can be destroyed resulting in blank ultrasound images. Consequentially, this method can promote the use of multiple sequential vascular-targeted bubble formulations with varying targets. This analysis, in turn, could result in the detailed quantification of the expression levels of biomarkers as disease progresses. Echogenic immuno-liposomes have been used based on this technique in efforts to develop an understanding of vascular receptor expression levels in atherosclerosis [203,204].

5.6 Particles for multimodal imaging

In the recent past, nanoparticle imaging agents have been developed for multimodal imaging. For instance, optical imaging and PET can be complementary to each other. A multimodal targeted nanoparticle can enable the use of PET for diagnostic purposes (*e.g.*, surgery planning) and the use of optical imaging during therapeutic interventions (*i.e.*, surgery). Along those lines, an MR-detectable and fluorescent liposome was developed for MR imaging and fluorescence microscopy [208]. By targeting $\alpha_v\beta_3$ integrins, multimodal molecular imaging facilitated detection of activated endothelium and angiogenic blood vessels. A different application of multimodal molecular imaging is the development of a ^{99m}Tc -Gd nanoparticles targeting $\alpha_v\beta_3$ integrins for molecular imaging of angiogenesis. The MR modality facilitates molecular imaging of angiogenesis with reasonable anatomical details, while the high sensitivity (but lower resolution) SPECT achieved detection of small lesions that were missed by MRI [209].

In the case of atherosclerosis, PET/CT [207,208], SPECT/CT [191], and CT/MRI/optical imaging [160] has been used to target macrophages at plaque sites. For the purposes of nanoparticle vascular targeting, VCAM-1-targeted particles conjugated to NIR probes have been used for dual MRI/optical imaging [145,147,191]. Similarly, ICAM-1 has also been used for multimodal particle targeting and imaging [190].

5.7 Considerations for clinical translation

In the previous sections, we reviewed a variety of nanoparticles designed for the most popular modalities used for molecular imaging: nuclear imaging, optical imaging, CT, MRI, and ultrasound. Table 1 illustrates that these imaging modalities present significantly different spatial resolution, penetration depth, contrast sensitivity and cost, which should be carefully considered to identify the most appropriate modality for a specific application.

While the predominant drive in academic research is and will continue to be scientific discovery and novelty, the successful development of a molecular imaging agent should carefully consider its bench-to-bedside translation leading to improved patient outcomes. In this context, the early-stage preclinical development of imaging agents needs to be mindful of aspects related to clinical adaptation and regulatory approval that extend well beyond successful imaging of overexpressed biomarkers in animal models. Such aspects include: 1) a well-defined unmet clinical need that the imaging agent addresses, 2) preclinical toxicity and safety, 3) identification of the primary clinical indication. Additionally, due to the complexity of manufacturing and controls (CMC) for clinical-grade nanoparticles, an early-stage analysis of the overall cost of product development can provide justification in terms of added benefits in terms of overall clinical impact. Further, contrary to conventional low molecular weight compounds, nanoparticle imaging agents present new safety considerations due to different *in vivo* pharmacokinetics and distribution. Additionally, the safety requirement for imaging agents is very high, since the target populations can be healthy subjects or low-risk cancer patients. This consideration makes evaluation of the benefit-to-risk ratio even more important.

Taking under consideration the manufacturing and safety requirements, radionuclide-based molecular imaging often provides the highest clinical relevance for widespread use in the clinic. While the resolution of either SPECT or PET (>1 mm) is not very high, radionuclide imaging provides high sensitivity, deep-tissue penetration, and quantitative nature. Clearly, the major advantage of SPECT and PET over other modalities (*e.g.*, CT, MRI) is their extraordinary sensitivity (down to the picomolar level). Thus, in addition to the diagnostic metrics of sensitivity and specificity, toxicity concerns of nanoparticle radiotracers are often alleviated, since tracer amounts are needed for radionuclide-based molecular imaging.

In summary, demonstration of clinical effectiveness strongly depends on the selection of the appropriate nanoparticle, imaging modality and clinical indication, which can then lead to substantial improvement in patient management. For instance, identification of the appropriate targets for ‘quantitative’ molecular imaging of plaque vulnerability can provide valuable guidance to the clinician for managing individual patients and preventing plaque rupture. As discussed in previous sections, representative targets of atherosclerotic features related to plaque vulnerability include neovascularization and plaque inflammation.

Furthermore, the combination of vascular targeting and nanoparticle imaging agents can lead to significant improvements in management of cancer patients. The concept of occult disease or “micrometastasis” emphasizes that when cancer patients have distant micrometastatic disease at the time of diagnosis, their risk for later development of overt metastatic disease is very high. Unfortunately, today’s imaging techniques can rarely detect micrometastases, which provides significant opportunities for molecular imaging. Various examples were provided in section 5 showing precise targeting of metastasis-associated vascular bed using nanoparticle imaging agents. Micrometastasis is defined as cohesive deposits of tumor cells of 2 mm or less but larger than 0.2 mm, which do not provide sufficient anatomical abnormalities to be imaged by standard MR or CT. Numerous retrospective analyses with long-term follow-up have shown an increased risk of breast cancer-related death in women with micrometastasis. Since the purpose of systemic therapy is typically to eradicate distant micrometastases, it is essential to evaluate the risk of an individual patient of harboring clinically silent micrometastatic disease. It is equally important to be able to select the optimal adjuvant or neoadjuvant therapy for an individual patient based on accurate diagnostic testing. While it is standard practice to administer systemic therapy to all patients with node-positive disease, it is clear that non-invasive and quantitative detection of lymph node metastasis or micrometastatic disease in distant organs may warrant more aggressive therapeutic approaches. In more advanced disease scenarios, nanoparticle-based molecular imaging can be developed for guiding treatment decisions around locoregional versus systemic treatment.

6. Conclusions

In conclusion, this review highlights the enormous potential of nanoparticles for molecular imaging applications due to multivalency, binding avidity and overall specificity and sensitivity of nanoparticles. It is further apparent that the natural synergy with vascular targeting can provide new avenues for diagnosis and prognosis of disease with quantitative precision. In addition to the obvious applications of targeting the molecular make-up of vascular diseases (*e.g.*, atherosclerosis), manifestation of other diseases, such cancer metastasis, initially occur on the endothelium providing opportunities for early detection and characterization of disease that are currently undetectable by today’s imaging techniques. Furthermore, most diseases continuously alter and remodel the endothelium providing a wide range of targets for molecular imaging, which will expand the diagnostic and prognostic value of imaging beyond anatomical and functional information. Thus, the prospect of added value in terms of improved patient management and outcomes justifies the extra complexity of nanoparticles’ fabrication, cost and regulatory procedures.

While the promise of targeted nanoparticles and molecular imaging is unquestionable, the field has suffered from poorly designed preclinical studies that lack clear clinical relevance or a well-defined endpoint. However, the field is maturing and preclinical development of nanotechnologies is increasingly becoming mindful of the clinical deployment even at the early stages of research, which can lead to molecular imaging that provides valuable assistance to clinicians and informs their decision-making process.

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References

1. Matsumura Y, Maeda H. A New Concept for Macromolecular Therapeutics in Cancer Chemotherapy: Mechanism of Tumoritropic Accumulation of Proteins and the Antitumor Agent SMANCS. *Cancer Res.* 1986; 46:6387–6392. [PubMed: 2946403]
2. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J. Control. Release.* 2000; 65:271–284. [PubMed: 10699287]
3. Maeda H, Tsukigawa K, Fang J. A Retrospective 30 Years After Discovery of the Enhanced Permeability and Retention Effect of Solid Tumors: Next-Generation Chemotherapeutics and Photodynamic Therapy—Problems, Solutions, and Prospects. *Microcirculation.* 2016; 23:173–182. [PubMed: 26237291]
4. Maeda H. The link between infection and cancer: Tumor vasculature, free radicals, and drug delivery to tumors via the EPR effect. *Cancer Sci.* 2013; 104:779–789. [PubMed: 23495730]
5. Nagamitsu A, Greish K, Maeda H. Elevating Blood Pressure as a Strategy to Increase Tumor-targeted Delivery of Macromolecular Drug SMANCS: Cases of Advanced Solid Tumors. *Jpn. J. Clin. Oncol.* 2009:074.
6. Maeda H. Vascular permeability in cancer and infection as related to macromolecular drug delivery, with emphasis on the EPR effect for tumor-selective drug targeting. *Proc. Jpn. Acad. Ser. B.* 2012; 88:53–71. [PubMed: 22450535]
7. Maeda H. Toward a full understanding of the EPR effect in primary and metastatic tumors as well as issues related to its heterogeneity. *Adv. Drug Deliv. Rev.* 2015; 91:3–6. [PubMed: 25579058]
8. Seki T, Fang J, Maeda H. Enhanced delivery of macromolecular antitumor drugs to tumors by nitroglycerin application. *Cancer Sci.* 2009; 100:2426–2430. [PubMed: 19793083]
9. Maeda H, Nakamura H, Fang J. The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. *Adv. Drug Deliv. Rev.* 2013; 65:71–79. [PubMed: 23088862]
10. Maeda H. The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. *Adv. Enzyme Regul.* 2001; 41:189–207. [PubMed: 11384745]
11. Nakamura H, Jun F, Maeda H. Development of next-generation macromolecular drugs based on the EPR effect: challenges and pitfalls. *Expert Opin. Drug Deliv.* 2015; 12:53–64. [PubMed: 25425260]
12. Maeda H. Research Spotlight: Emergence of EPR effect theory and development of clinical applications for cancer therapy. *Ther. Deliv.* 2014; 5:627–630. [PubMed: 25090276]
13. Fang J, Liao L, Yin H, Nakamura H, Subr V, Ulbrich K, Maeda H. Photodynamic therapy and imaging based on tumor-targeted nanoprobe, polymer-conjugated zinc protoporphyrin. *Future Sci. OA.* 2015; 1
14. Park K. Questions on the role of the EPR effect in tumor targeting. *J. Control. Release.* 2013; 172:391. [PubMed: 24113486]
15. Dreher MR, Liu W, Michelich CR, Dewhirst MW, Yuan F, Chilkoti A. Tumor Vascular Permeability, Accumulation, and Penetration of Macromolecular Drug Carriers. *J. Natl. Cancer Inst.* 2006; 98:335–344. [PubMed: 16507830]
16. Pluen A, Boucher Y, Ramanujan S, McKee TD, Gohongi T, di Tomaso E, Brown EB, Izumi Y, Campbell RB, Berk DA, Jain RK. Role of tumor–host interactions in interstitial diffusion of macromolecules: Cranial vs. subcutaneous tumors. *Proc. Natl. Acad. Sci.* 2001; 98:4628–4633. [PubMed: 11274375]

17. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144:646–674. [PubMed: 21376230]
18. Neri D, Bicknell R. Tumour vascular targeting. *Nat. Rev. Cancer*. 2005; 5:436–446. [PubMed: 15928674]
19. Bae YH, Park K. Targeted drug delivery to tumors: Myths, reality and possibility. *J. Control. Release*. 2011; 153:198–205. [PubMed: 21663778]
20. Ruoslahti E, Bhatia SN, Sailor MJ. Targeting of drugs and nanoparticles to tumors. *J. Cell Biol*. 2010; 188:759–768. [PubMed: 20231381]
21. Jain RK. Transport of Molecules, Particles, and Cells in Solid Tumors. *Annu. Rev. Biomed. Eng*. 1999; 1:241–263. [PubMed: 11701489]
22. McDonald DM, Choyke PL. Imaging of angiogenesis: from microscope to clinic. *Nat. Med*. 2003; 9:713–725. [PubMed: 12778170]
23. Ma K, Aj M, A L. Microvascular corrosion casting in the study of tumor vascularity: a review. *Scanning Microsc*. 1994; 9:1243–1244.
24. Skinner SA, Tutton PJM, O'Brien PE. Microvascular Architecture of Experimental Colon Tumors in the Rat. *Cancer Res*. 1990; 50:2411–2417. [PubMed: 2317825]
25. Yuan F, Dellian M, Fukumura D, Leunig M, Berk DA, Torchilin VP, Jain RK. Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. *Cancer Res*. 1995; 55:3752–3756. [PubMed: 7641188]
26. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol*. 2007; 2:751–760. [PubMed: 18654426]
27. Gerlowski LE, Jain RK. Microvascular permeability of normal and neoplastic tissues. *Microvasc. Res*. 1986; 31:288–305. [PubMed: 2423854]
28. Stewart JM, Whalley ET, Cheronis JC, Maeda H, Akaike T, Wu J, Noguchi Y, Sakata Y. Bradykinin and nitric oxide in infectious disease and cancer. *Immunopharmacology*. 1996; 33:222–230. [PubMed: 8856154]
29. Maeda H. Modified Therapeutic Proteins SMANCS and polymer-conjugated macromolecular drugs: advantages in cancer chemotherapy. *Adv. Drug Deliv. Rev*. 1991; 6:181–202.
30. Noguchi Y, Wu J, Duncan R, Strohalm J, Ulbrich K, Akaike T, Maeda H. Early Phase Tumor Accumulation of Macromolecules: A Great Difference in Clearance Rate between Tumor and Normal Tissues. *Jpn. J. Cancer Res*. 1998; 89:307–314. [PubMed: 9600125]
31. Maeda H, Noguchi Y, Sato K, Akaike T. Enhanced Vascular Permeability in Solid Tumor Is Mediated by Nitric Oxide and Inhibited by Both New Nitric Oxide Scavenger and Nitric Oxide Synthase Inhibitor. *Jpn. J. Cancer Res*. 1994; 85:331–334. [PubMed: 7515384]
32. Kiessling F, Mertens ME, Grimm J, Lammers T. Nanoparticles for Imaging: Top or Flop? *Radiology*. 2014; 273:10–28. [PubMed: 25247562]
33. Arap W, Pasqualini R, Ruoslahti E. Cancer Treatment by Targeted Drug Delivery to Tumor Vasculature in a Mouse Model. *Science*. 1998; 279:377–380. [PubMed: 9430587]
34. Cai W, Gambhir SS, Chen X. Chapter 7. Molecular imaging of tumor vasculature. *Methods Enzymol*. 2008; 445:141–176. [PubMed: 19022059]
35. Pysz MA, Foygel K, Rosenberg J, Gambhir SS, Schneider M, Willmann JK. Antiangiogenic Cancer Therapy: Monitoring with Molecular US and a Clinically Translatable Contrast Agent (BR55). *Radiology*. 2010; 256:519–527. [PubMed: 20515975]
36. Dudzinski DM, Michel T. Life history of eNOS: Partners and pathways. *Cardiovasc. Res*. 2007; 75:247–260. [PubMed: 17466957]
37. Michel T, Feron O. Nitric oxide synthases: which, where, how, and why? *J. Clin. Invest*. 1997; 100:2146–2152. [PubMed: 9410890]
38. Wu J, Akaike T, Hayashida K, Okamoto T, Okuyama A, Maeda H. Enhanced Vascular Permeability in Solid Tumor Involving Peroxynitrite and Matrix Metalloproteinases. *Jpn. J. Cancer Res*. 2001; 92:439–451. [PubMed: 11346467]
39. Barter P, Gotto AM, LaRosa JC, Maroni J, Szarek M, Grundy SM, Kastelein JJP, Bittner V, Fruchart J-C. HDL Cholesterol, Very Low Levels of LDL Cholesterol, and Cardiovascular Events. *N. Engl. J. Med*. 2007; 357:1301–1310. [PubMed: 17898099]

40. Ross R. Atherosclerosis — An Inflammatory Disease. *N. Engl. J. Med.* 1999; 340:115–126. [PubMed: 9887164]
41. Libby P, Ridker PM, Maseri A. Inflammation and Atherosclerosis. *Circulation.* 2002; 105:1143.
42. Blankenberg S, Barbaux S, Tiret L. Adhesion molecules and atherosclerosis. *Atherosclerosis.* 2003; 170:191–203. [PubMed: 14612198]
43. Hashizume H, Baluk P, Morikawa S, McLean JW, Thurston G, Roberge S, Jain RK, McDonald DM. Openings between Defective Endothelial Cells Explain Tumor Vessel Leakiness. *Am. J. Pathol.* 2000; 156:1363–1380. [PubMed: 10751361]
44. Tabas I. Macrophage death and defective inflammation resolution in atherosclerosis. *Nat. Rev. Immunol.* 2010; 10:36–46. [PubMed: 19960040]
45. Arroyo LH, Lee RT. Mechanisms of plaque rupture. *Cardiovasc. Res.* 1999; 41:369–375. [PubMed: 10341836]
46. Ritman EL, Lerman A. The Dynamic Vasa Vasorum. *Cardiovasc. Res.* 2007; 75:649–658. [PubMed: 17631284]
47. Winter PM, Neubauer AM, Caruthers SD, Harris TD, Robertson JD, Williams TA, Schmieder AH, Hu G, Allen JS, Lacy EK, Zhang H, Wickline SA, Lanza GM. Endothelial $\alpha v \beta 3$ Integrin–Targeted Fumagillin Nanoparticles Inhibit Angiogenesis in Atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 2006; 26:2103–2109. [PubMed: 16825592]
48. Jaffer FA, Libby P, Weissleder R. Molecular and Cellular Imaging of Atherosclerosis Emerging Applications. *J. Am. Coll. Cardiol.* 2006; 47:1328–1338. [PubMed: 16580517]
49. Rudd JHF, Warburton EA, Fryer TD, Jones HA, Clark JC, Antoun N, Johnström P, Davenport AP, Kirkpatrick PJ, Arch BN, Pickard JD, Weissberg PL. Imaging Atherosclerotic Plaque Inflammation With [18F]-Fluorodeoxyglucose Positron Emission Tomography. *Circulation.* 2002; 105:2708–2711. [PubMed: 12057982]
50. Zhang Z, Machac J, Helft G, Worthley SG, Tang C, Zaman AG, Rodriguez OJ, Buchsbaum MS, Fuster V, Badimon JJ. Non-invasive imaging of atherosclerotic plaque macrophage in a rabbit model with F-18 FDG PET: a histopathological correlation. *BMC Nucl. Med.* 2006; 6:3. [PubMed: 16725052]
51. Tarkin JM, Dweck MR, Evans NR, Takx RAP, Brown AJ, Tawakol A, Fayad ZA, Rudd JHF. Imaging Atherosclerosis. *Circulation Res.* 2015; 118:750–769.
52. Stendahl JC, Sinusas AJ. Nanoparticles for Cardiovascular Imaging and Therapeutic Delivery, Part 1: Compositions and Features. *J. Nucl. Med.* 2015; 56:1469–1475. [PubMed: 26272808]
53. Stendahl JC, Sinusas AJ. Nanoparticles for Cardiovascular Imaging and Therapeutic Delivery, Part 2: Radiolabeled Probes. *J. Nucl. Med.* 2015; 56:1637–1641. [PubMed: 26294304]
54. Guyton, AC., Hall, JE. *Textbook of Medical Physiology.* 11th. Philadelphia: Elsevier, Inc.; 2006.
55. Hoeben A, Landuyt B, Highley MS, Wildiers H, Oosterom ATV, Bruijn EAD. Vascular Endothelial Growth Factor and Angiogenesis. *Pharmacol. Rev.* 2004; 56:549–580. [PubMed: 15602010]
56. Hanahan D, Weinberg RA. The Hallmarks of Cancer. *Cell.* 2000; 100:57–70. [PubMed: 10647931]
57. Suzuki M, Takahashi T, Sato T. Medial regression and its functional significance in tumor-supplying host arteries. A morphometric study of hepatic arteries in human livers with hepatocellular carcinoma. *Cancer.* 1987; 59:444–450. [PubMed: 3024804]
58. Suzuki M, Hori K, Abe I, Saito S, Sato H. A New Approach to Cancer Chemotherapy: Selective Enhancement of Tumor Blood Flow With Angiotensin II. *J. Nat. Cancer Inst.* 1981; 67:663–669. [PubMed: 6944536]
59. Carmeliet P, Jain RK. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. *Nat. Rev. Drug Discov.* 2011; 10:417–427. [PubMed: 21629292]
60. Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. *Nat. Rev. Clin. Oncol.* 2010; 7:653–664. [PubMed: 20838415]
61. Toy R, Hayden E, Camann A, Berman Z, Vicente P, Tran E, Meyers J, Pansky J, Peiris PM, Wu H, Exner A, Wilson D, Ghaghada KB, Karathanasis E. Multimodal In Vivo Imaging Exposes the Voyage of Nanoparticles in Tumor Microcirculation. *ACS Nano.* 2013; 7:3118–3129. [PubMed: 23464827]

62. Chiu J-J, Chien S. Effects of Disturbed Flow on Vascular Endothelium: Pathophysiological Basis and Clinical Perspectives. *Physiol. Rev.* 2011; 91:327–387. [PubMed: 21248169]
63. Stone PH, Saito S, Takahashi S, Makita Y, Nakamura S, Kawasaki T, Takahashi A, Katsuki T, Nakamura S, Namiki A, Hirohata A, Matsumura T, Yamazaki S, Yokoi H, Tanaka S, Otsuji S, Yoshimachi F, Honye J, Harwood D, Reitman M, Coskun AU, Papafaklis MI, Feldman CL. Prediction of Progression of Coronary Artery Disease and Clinical Outcomes Using Vascular Profiling of Endothelial Shear Stress and Arterial Plaque Characteristics: The PREDICTION Study. *Circulation.* 2012; 126:172–181. [PubMed: 22723305]
64. Toy R, Bauer L, Hoimes C, Ghaghada KB, Karathanasis E. Targeted nanotechnology for cancer imaging. *Adv. Drug Deliv. Rev.* 2014; 76:79–97. [PubMed: 25116445]
65. Re F, Moresco R, Masserini M. Nanoparticles for neuroimaging. *J. Phys. Appl. Phys.* 2012; 45:073001.
66. Toy R, Peiris PM, Ghaghada KB, Karathanasis E. Shaping cancer nanomedicine: the effect of particle shape on the in vivo journey of nanoparticles. *Nanomed.* 2013; 9:121–134.
67. Piner RD, Zhu J, Xu F, Hong S, Mirkin CA. 'Dip-Pen' Nanolithography. *Science.* 1999; 283:661–663. [PubMed: 9924019]
68. Canelas DA, Herlihy KP, DeSimone JM. Top-down particle fabrication: control of size and shape for diagnostic imaging and drug delivery. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 2009; 1:391–404. [PubMed: 20049805]
69. Toy R, Hayden E, Shoup C, Baskaran H, Karathanasis E. The effects of particle size, density and shape on margination of nanoparticles in microcirculation. *Nanotechnology.* 2011; 22:115101. [PubMed: 21387846]
70. Choi HS, Liu W, Liu F, Nasr K, Misra P, Bawendi MG, Frangioni JV. Design considerations for tumour-targeted nanoparticles. *Nat. Nanotechnol.* 2010; 5:42–47. [PubMed: 19893516]
71. Owens DE III, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *Int. J. Pharm.* 2006; 307:93–102. [PubMed: 16303268]
72. Moghimi SM, Szebeni J. Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties. *Prog. Lipid Res.* 2003; 42:463–478. [PubMed: 14559067]
73. Litzinger DC, Buiting AMJ, van Rooijen N, Huang L. Effect of liposome size on the circulation time and intraorgan distribution of amphipathic poly(ethylene glycol)-containing liposomes. *Biochim. Biophys. Acta BBA - Biomembr.* 1994; 1190:99–107.
74. Nagayasu A, Uchiyama K, Kiwada H. The size of liposomes: a factor which affects their targeting efficiency to tumors and therapeutic activity of liposomal antitumor drugs. *Adv. Drug Deliv. Rev.* 1999; 40:75–87. [PubMed: 10837781]
75. Ma X, Wu Y, Jin S, Tian Y, Zhang X, Zhao Y, Yu L, Liang X-J. Gold Nanoparticles Induce Autophagosome Accumulation through Size-Dependent Nanoparticle Uptake and Lysosome Impairment. *ACS Nano.* 2011; 5:8629–8639. [PubMed: 21974862]
76. Chithrani BD, Ghazani AA, Chan WCW. Determining the Size and Shape Dependence of Gold Nanoparticle Uptake into Mammalian Cells. *Nano Lett.* 2006; 6:662–668. [PubMed: 16608261]
77. Kibria G, Hatakeyama H, Ohga N, Hida K, Harashima H. The effect of liposomal size on the targeted delivery of doxorubicin to Integrin $\alpha v \beta 3$ -expressing tumor endothelial cells. *Biomaterials.* 2013; 34:5617–5627. [PubMed: 23623323]
78. Champion JA, Mitragotri S. Role of target geometry in phagocytosis. *Proc. Natl. Acad. Sci. U. S. A.* 2006; 103:4930–4934. [PubMed: 16549762]
79. Arnida M, Janát-Amsbury M, Ray A, Peterson CM, Ghandehari H. Geometry and surface characteristics of gold nanoparticles influence their biodistribution and uptake by macrophages. *Eur. J. Pharm. Biopharm.* 2011; 77:417–442. [PubMed: 21093587]
80. Huang X, Li L, Liu T, Hao N, Liu H, Chen D, Tang F. The Shape Effect of Mesoporous Silica Nanoparticles on Biodistribution, Clearance, and Biocompatibility in Vivo. *ACS Nano.* 2011; 5:5390–5399. [PubMed: 21634407]
81. Gavze E, Shapiro M. Particles in a shear flow near a solid wall: Effect of nonsphericity on forces and velocities. *Int. J. Multiph. Flow.* 23:155–182. (1997).

82. Adriani G, de Tullio MD, Ferrari M, Hussain F, Pascazio G, Liu X, Decuzzi P. The preferential targeting of the diseased microvasculature by disk-like particles. *Biomaterials*. 2012; 33:5504–5513. [PubMed: 22579236]
83. Doshi N, Prabhakarparandian B, Rea-Ramsey A, Pant K, Sundaram S, Mitragotri S. Flow and adhesion of drug carriers in blood vessels depend on their shape: A study using model synthetic microvascular networks. *J. Control. Release*. 2010; 146:196–200. [PubMed: 20385181]
84. Lee S-Y, Ferrari M, Decuzzi P. Shaping nano-/micro-particles for enhanced vascular interaction in laminar flows. *Nanotechnology*. 2009; 20:495101. [PubMed: 19904027]
85. Peiris PM, Toy R, Doolittle E, Pansky J, Abramowski A, Tam M, Vicente P, Tran E, Hayden E, Camann A, Mayer A, Erokwu BO, Berman Z, Wilson D, Baskaran H, Flask CA, Keri RA, Karathanasis E. Imaging Metastasis Using an Integrin-Targeting Chain-Shaped Nanoparticle. *ACS Nano*. 2012; 6:8783–8795. [PubMed: 23005348]
86. Peiris PM, Schmidt E, Calabrese M, Karathanasis E. Assembly of Linear Nano-Chains from Iron Oxide Nanospheres with Asymmetric Surface Chemistry. *PLoS ONE*. 2011; 6:e15927. [PubMed: 21253600]
87. Peiris PM, Bauer L, Toy R, Tran E, Pansky J, Doolittle E, Schmidt E, Hayden E, Mayer A, Keri RA, Griswold MA, Karathanasis E. Enhanced Delivery of Chemotherapy to Tumors Using a Multicomponent Nanochain with Radio-Frequency-Tunable Drug Release. *ACS Nano*. 2012; 6:4157–4168. [PubMed: 22486623]
88. Peiris PM, Tam M, Vicente P, Abramowski A, Toy R, Bauer L, Mayer A, Pansky J, Doolittle E, Tucci S, Schmidt E, Shoup C, Rao S, Murray K, Gopalakrishnan R, Keri RA, Basilion JP, Griswold MA, Karathanasis E. On-Command Drug Release from Nanochains Inhibits Growth of Breast Tumors. *Pharm. Res*. 2014; 31:1460–1468. [PubMed: 23934254]
89. Peiris PM, Toy R, Abramowski A, Vicente P, Tucci S, Bauer L, Mayer A, Tam M, Doolittle E, Pansky J, Tran E, Lin D, Schiemann WP, Ghaghada KB, Griswold MA, Karathanasis E. Treatment of cancer micrometastasis using a multicomponent chain-like nanoparticle. *J. Control. Release*. 2014; 173:51–58. [PubMed: 24188960]
90. Peiris PM, Abramowski A, McGinnity J, Doolittle E, Toy R, Gopalakrishnan R, Shah S, Bauer L, Ghaghada KB, Hoimes C, Brady-Kalnay SM, Basilion JP, Griswold MA, Karathanasis E. Treatment of invasive brain tumors using a chain-like nanoparticle. *Cancer Res*. 2015; 75:1356–1365. [PubMed: 25627979]
91. Peiris PM, Deb P, Doolittle E, Doron G, Goldberg A, Govender P, Shah S, Rao S, Carbone S, Cotey T, Sylvestre M, Singh S, Schiemann WP, Lee Z, Karathanasis E. Vascular Targeting of a Gold Nanoparticle to Breast Cancer Metastasis. *J. Pharm. Sci*. 2015; 104:2600–2610. [PubMed: 26036431]
92. Kolhar P, Anselmo AC, Gupta V, Pant K, Prabhakarparandian B, Ruoslahti E, Mitragotri S. Using shape effects to target antibody-coated nanoparticles to lung and brain endothelium. *Proc. Natl. Acad. Sci*. 2013; 110:10753–10758. [PubMed: 23754411]
93. Shuvaev VV, Ilies MA, Simone E, Zaitsev S, Kim Y, Cai S, Mahmud A, Dziubla T, Muro S, Discher DE, Muzykantov VR. Endothelial Targeting of Antibody-Decorated Polymeric Filomicelles. *ACS Nano*. 2011; 5:6991–6999. [PubMed: 21838300]
94. Valencia PM, Pridgen EM, Rhee M, Langer R, Farokhzad OC, Karnik R. Microfluidic Platform for Combinatorial Synthesis and Optimization of Targeted Nanoparticles for Cancer Therapy. *ACS Nano*. 2013; 7:10671–10680. [PubMed: 24215426]
95. Stefanick JF, Ashley JD, Kiziltepe T, Bilgic B. A Systematic Analysis of Peptide Linker Length and Liposomal Polyethylene Glycol Coating on Cellular Uptake of Peptide-Targeted Liposomes. *ACS Nano*. 2013; 7:2935–2947. [PubMed: 23421406]
96. Ghaghada KB, Saul J, Natarajan JV, Bellamkonda RV, Annapragada AV. Folate targeting of drug carriers: A mathematical model. *J. Control. Release*. 2005; 104:113–128. [PubMed: 15866339]
97. Doolittle E, Peiris PM, Doron G, Goldberg A, Tucci S, Rao S, Shah S, Sylvestre M, Govender P, Turan O, Lee Z, Schiemann WP, Karathanasis E. Spatiotemporal Targeting of a Dual-Ligand Nanoparticle to Cancer Metastasis. *ACS Nano*. 2015; 9:8012–8021. [PubMed: 26203676]
98. Cai W, Chen X. Multimodality Molecular Imaging of Tumor Angiogenesis. *J. Nucl. Med*. 2008; 49(Suppl 2):113S–128S. [PubMed: 18523069]

99. Li S, Peck-Radosavljevic M, Kienast O, Preitfellner J, Hamilton G, Kurtaran A, Pirich C, Angelberger P, Dudczak R. Imaging gastrointestinal tumours using vascular endothelial growth factor-165 (VEGF165) receptor scintigraphy. *Ann. Oncol.* 2003; 14:1274–1277. [PubMed: 12881392]
100. Li S, Peck-Radosavljevic M, Kienast O, Preitfellner J, et al. Iodine-123-vascular endothelial growth factor-165 (¹²³I-VEGF₁₆₅) Biodistribution, safety and radiation dosimetry in patients with pancreatic carcinoma. *Q. J. Nucl. Med. Mol. Imaging.* 2004; 48:198–206. [PubMed: 15499293]
101. Yoshimoto M, Kinuya S, Kawashima A, Nishii R, Yokoyama K, Kawai K. Radioiodinated VEGF to image tumor angiogenesis in a LS180 tumor xenograft model. *Nucl. Med. Biol.* 2006; 33:963–969. [PubMed: 17127168]
102. Backer MV, Levashova Z, Patel V, Jehning BT, Claffey K, Blankenberg FG, Backer JM. Molecular imaging of VEGF receptors in angiogenic vasculature with single-chain VEGF-based probes. *Nat. Med.* 2007; 13:504–509. [PubMed: 17351626]
103. Blankenberg FG, Backer MV, Levashova Z, Patel V, Backer JM. In vivo tumor angiogenesis imaging with site-specific labeled 99mTc-HYNIC-VEGF. *Eur. J. Nucl. Med. Mol. Imaging.* 2006; 33:841–848. [PubMed: 16699765]
104. Blankenberg FG, Mandl S, Cao Y-A, O'Connell-Rodwell C, Contag C, Mari C, Gaynutdinov TI, Vanderheyden J-L, Backer MV, Backer JM. Tumor Imaging Using a Standardized Radiolabeled Adapter Protein Docked to Vascular Endothelial Growth Factor. *J. Nucl. Med.* 2004; 45:1373–1380. [PubMed: 15299064]
105. Collingridge DR, Carroll VA, Glaser M, Aboagye EO, Osman S, Hutchinson OC, Barthel H, Luthra SK, Brady F, Bicknell R, Price P, Harris AL. The Development of [124I]Iodinated-VG766 A Novel Tracer for Imaging Vascular Endothelial Growth Factor in Vivo Using Positron Emission Tomography. *Cancer Res.* 2002; 62:5912–5919. [PubMed: 12384557]
106. Nagengast WB, de Vries EG, Hospers GA, Mulder NH, de Jong JR, Hollema H, Brouwers AH, van Dongen GA, Perk LR, Hooze MNL. In Vivo VEGF Imaging with Radiolabeled Bevacizumab in a Human Ovarian Tumor Xenograft. *J. Nucl. Med.* 2007; 48:1313–1319. [PubMed: 17631557]
107. Liu Z, Cai W, He L, Nakayama N, Chen K, Sun X, Chen X, Dai H. In vivo biodistribution and highly efficient tumour targeting of carbon nanotubes in mice. *Nat. Nanotechnol.* 2007; 2:47–52. [PubMed: 18654207]
108. Kim Y-H, Jeon J, Hong SH, Rhim W-K, Lee Y-S, Youn H, Chung J-K, Lee MC, Lee DS, Kang KW, Nam J-M. Tumor Targeting and Imaging Using Cyclic RGD-PEGylated Gold Nanoparticle Probes with Directly Conjugated Iodine-125. *Small.* 2011; 7:2052–2060. [PubMed: 21688390]
109. Tian M, Lu W, Zhang R, Xiong C, Ensor J, Nazario J, Jackson J, Shaw C, Dixon KA, Miller J, Wright K, Li C, Gupta S. Tumor Uptake of Hollow Gold Nanospheres After Intravenous and Intra-arterial Injection: PET/CT Study in a Rabbit VX2 Liver Cancer Model. *Mol. Imaging Biol.* 2013; 15:614–624. [PubMed: 23608932]
110. Chen F, Hong H, Zhang Y, Valdovinos HF, Shi S, Kwon GS, Theuer CP, Barnhart TE, Cai W. In Vivo Tumor Targeting and Image-Guided Drug Delivery with Antibody-Conjugated, Radiolabeled Mesoporous Silica Nanoparticles. *ACS Nano.* 2013; 7:9027–9039. [PubMed: 24083623]
111. Broisat A, Toczek J, Dumas LS, Ahmadi M, Bacot S, Perret P, Slimani L, Barone-Rochette G, Soubies A, Devoogdt N, Lahoutte T, Fagret D, Riou LM, Ghezzi C. 99mTc-cAbVCAM1–5 Imaging Is a Sensitive and Reproducible Tool for the Detection of Inflamed Atherosclerotic Lesions in Mice. *J. Nucl. Med.* 2014; 55:1678–1684. [PubMed: 25157043]
112. Sipkins DA, Wei X, Wu JW, Runnels JM, Côté D, Means TK, Luster AD, Scadden DT, Lin CP. In vivo imaging of specialized bone marrow endothelial microdomains for tumour engraftment. *Nature.* 2005; 435:969–973. [PubMed: 15959517]
113. Luo S, Zhang E, Su Y, Cheng T, Shi C. A review of NIR dyes in cancer targeting and imaging. *Biomaterials.* 2011; 32:7127–7138. [PubMed: 21724249]
114. Setua S, Menon D, Asok A, Nair S, Koyakutty M. Folate receptor targeted, rare-earth oxide nanocrystals for bi-modal fluorescence and magnetic imaging of cancer cells. *Biomaterials.* 2010; 31:714–729. [PubMed: 19822364]

115. Hashizume H, Baluk P, Morikawa S, McLean JW, Thurston G, Roberge S, Jain RK, McDonald DM. Openings between Defective Endothelial Cells Explain Tumor Vessel Leakiness. *Am. J. Pathol.* 2000; 156:1363–1380. [PubMed: 10751361]
116. Thurston G, McLean JW, Rizen M, Baluk P, Haskell A, Murphy TJ, Hanahan D, McDonald DM. Cationic liposomes target angiogenic endothelial cells in tumors and chronic inflammation in mice. *J. Clin. Invest.* 1998; 101:1401–1413. [PubMed: 9525983]
117. McLean JW, Fox EA, Baluk P, Bolton PB, Haskell A, Pearlman R, Thurston G, Umemoto EY, McDonald DM. Organ-specific endothelial cell uptake of cationic liposome-DNA complexes in mice. *Am J Physiol.* 1997; 273:H387–H404. [PubMed: 9249514]
118. Pasqualini R, Arap W, McDonald DM. Probing the structural and molecular diversity of tumor vasculature. *Trends Mol. Med.* 2002; 8:563–571. [PubMed: 12470989]
119. Koivunen E, Wang B, Ruoslahti E. Phage Libraries Displaying Cyclic Peptides with Different Ring Sizes: Ligand Specificities of the RGD-Directed Integrins. *Bio/Technology.* 1995; 13:265–270. [PubMed: 9634769]
120. Pasqualini R, Koivunen E, Ruoslahti E. α_v Integrins as receptors for tumor targeting by circulating ligands. *Nat. Biotechnol.* 1997; 15:542–546. [PubMed: 9181576]
121. Lewis JD, Destito G, Zijlstra A, Gonzalez MJ, Quigley JP, Manchester M, Stuhlmann H. Viral nanoparticles as tools for intravital vascular imaging. *Nat. Med.* 2006; 12:354–360. [PubMed: 16501571]
122. Gargsha M, Qutaish MQ, Roy D, Steyer GJ, Watanabe M, Wilson DL. Visualization of color anatomy and molecular fluorescence in whole-mouse cryo-imaging. *Comput. Med. Imaging Graph.* 2011; 35:195–205. [PubMed: 21041065]
123. Qutaish MQ, Sullivant KE, Burden-Gulley SM, Lu H, Roy D, Wang J, Basilion JP, Brady-Kalnay SM, Wilson DL. Cryo-image Analysis of Tumor Cell Migration, Invasion, and Dispersal in a Mouse Xenograft Model of Human Glioblastoma Multiforme. *Mol. Imaging Biol. MIB Off. Publ. Acad. Mol. Imaging.* 2012; 14:572–583.
124. Burden-Gulley SM, Qutaish MQ, Sullivant KE, Lu H, Wang J, L. Craig SE, Basilion JP, Wilson DL, Brady-Kalnay SM. Novel Cryo-Imaging of the Glioma Tumor Microenvironment Reveals Migration and Dispersal Pathways in Vivid Three-Dimensional Detail. *Cancer Res.* 2011; 71:5932–5940. [PubMed: 21862632]
125. Zhou Z, Qutaish M, Han Z, Schur RM, Liu Y, Wilson DL, Lu Z-R. MRI detection of breast cancer micrometastases with a fibronectin-targeting contrast agent. *Nat. Commun.* 2015; 6:7984. [PubMed: 26264658]
126. Gao X, Cui Y, Levenson RM, Chung LWK, Nie S. In vivo cancer targeting and imaging with semiconductor quantum dots. *Nat. Biotechnol.* 2004; 22:969–976. [PubMed: 15258594]
127. Åkerman ME, Chan WCW, Laakkonen P, Bhatia SN, Ruoslahti E. Nanocrystal targeting in vivo. *Proc. Natl. Acad. Sci.* 2002; 99:12617–12621. [PubMed: 12235356]
128. Cai W, Shin D-W, Chen K, Gheysens O, Cao Q, Wang SX, Gambhir SS, Chen X. Peptide-Labeled Near-Infrared Quantum Dots for Imaging Tumor Vasculature in Living Subjects. *Nano Lett.* 2006; 6:669–676. [PubMed: 16608262]
129. Tada H, Higuchi H, Wanatabe TM, Ohuchi N. In vivo Real-time Tracking of Single Quantum Dots Conjugated with Monoclonal Anti-HER2 Antibody in Tumors of Mice. *Cancer Res.* 2007; 67:1138–1144. [PubMed: 17283148]
130. Montet X, Montet-Abou K, Reynolds F, Weissleder R, Josephson L. Nanoparticle Imaging of Integrins on Tumor Cells. *Neoplasia.* 2006; 8:214–222. [PubMed: 16611415]
131. Medintz IL, Uyeda HT, Goldman ER, Mattoussi H. Quantum dot bioconjugates for imaging, labelling and sensing. *Nat. Mater.* 2005; 4:435–446. [PubMed: 15928695]
132. Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sundaresan G, Wu AM, Gambhir SS, Weiss S. Quantum Dots for Live Cells, in Vivo Imaging, and Diagnostics. *Science.* 2005; 307:538–544. [PubMed: 15681376]
133. Li Z-B, Cai W, Chen X. Semiconductor Quantum Dots for In Vivo Imaging. *J. Nanosci. Nanotechnol.* 2007; 7:2567–2581. [PubMed: 17685272]

134. Gao J, Chen K, Luong R, Bouley DM, Mao H, Qiao T, Gambhir SS, Cheng Z. A Novel Clinically Translatable Fluorescent Nanoparticle for Targeted Molecular Imaging of Tumors in Living Subjects. *Nano Lett.* 2012; 12:281–286. [PubMed: 22172022]
135. Smith BR, Cheng Z, De A, Koh AL, Sinclair R, Gambhir SS. Real-Time Intravital Imaging of RGD–Quantum Dot Binding to Luminal Endothelium in Mouse Tumor Neovasculature. *Nano Lett.* 2008; 8:2599–2606. [PubMed: 18386933]
136. Smith BR, Kempen P, Bouley D, Xu A, Liu Z, Melosh N, Dai H, Sinclair R, Gambhir SS. Shape Matters: Intravital Microscopy Reveals Surprising Geometrical Dependence for Nanoparticles in Tumor Models of Extravasation. *Nano Lett.* 2012; 12:3369–3377. [PubMed: 22650417]
137. Aaron J, Nitin N, Travis K, Kumar S, Collier T, Park SY, José-Yacamán M, Coghlan L, Follen M, Richards-Kortum R, Sokolov K. Plasmon resonance coupling of metal nanoparticles for molecular imaging of carcinogenesis in vivo. *J. Biomed. Opt.* 2007; 12:034007–034011. [PubMed: 17614715]
138. El-Sayed IH, Huang X, El-Sayed MA. Surface Plasmon Resonance Scattering and Absorption of anti-EGFR Antibody Conjugated Gold Nanoparticles in Cancer Diagnostics: Applications in Oral Cancer. *Nano Lett.* 2005; 5:829–834. [PubMed: 15884879]
139. Skala MC, Crow MJ, Wax A, Izatt JA. Photothermal Optical Coherence Tomography of Epidermal Growth Factor Receptor in Live Cells Using Immunotargeted Gold Nanospheres. *Nano Lett.* 2008; 8:3461–3467. [PubMed: 18767886]
140. Durr NJ, Larson T, Smith DK, Korgel BA, Sokolov K, Ben-Yakar A. Two-Photon Luminescence Imaging of Cancer Cells Using Molecularly Targeted Gold Nanorods. *Nano Lett.* 2007; 7:941–945. [PubMed: 17335272]
141. Huang X, El-Sayed IH, Qian W, El-Sayed MA. Cancer Cell Imaging and Photothermal Therapy in the Near-Infrared Region by Using Gold Nanorods. *J. Am. Chem. Soc.* 2006; 128:2115–2120. [PubMed: 16464114]
142. Skrabalak SE, Chen J, Au L, Lu X, Li X, Xia Y. Gold Nanocages for Biomedical Applications. *Adv. Mater.* 2007; 19:3177–3184. [PubMed: 18648528]
143. Skrabalak SE, Chen J, Sun Y, Lu X, Au L, Cogley CM, Xia Y. Gold Nanocages: Synthesis, Properties, and Applications. *Acc. Chem. Res.* 2008; 41:1587–1595. [PubMed: 18570442]
144. Xia Y. Improving biomedical imaging with gold nanocages. *SPIE Newsroom.* 2008
145. Kelly KA, Allport JR, Tsourkas A, Shinde-Patil VR, Josephson L, Weissleder R. Detection of Vascular Adhesion Molecule-1 Expression Using a Novel Multimodal Nanoparticle. *Circ. Res.* 2005; 96:327–336. [PubMed: 15653572]
146. Morishige K, Kacher DF, Libby P, Josephson L, Ganz P, Weissleder R, Aikawa M. High-Resolution Magnetic Resonance Imaging Enhanced With Superparamagnetic Nanoparticles Measures Macrophage Burden in Atherosclerosis. *Circulation.* 2010; 122:1707–1715. [PubMed: 20937980]
147. Tsourkas A, Shinde-Patil VR, Kelly KA, Patel P, Wolley A, Allport JR, Weissleder R. In Vivo Imaging of Activated Endothelium Using an Anti-VCAM-1 Magnetooptical Probe. *Bioconjug. Chem.* 2005; 16:576–581. [PubMed: 15898724]
148. Michalska M, Machtoub L, Manthey HD, Bauer E, Herold V, Krohne G, Lykowsky G, Hildenbrand M, Kampf T, Jakob P, Zerneck A, Bauer WR. Visualization of Vascular Inflammation in the Atherosclerotic Mouse by Ultrasmall Superparamagnetic Iron Oxide Vascular Cell Adhesion Molecule-1–Specific Nanoparticles. *Arterioscler. Thromb. Vasc. Biol.* 2012; 32:2350–2357. [PubMed: 22879583]
149. Iwai K, Maeda H, Konno T. Use of Oily Contrast Medium for Selective Drug Targeting to Tumor: Enhanced Therapeutic Effect and X-Ray Image. *Cancer Res.* 1984; 44:2115–2121. [PubMed: 6324996]
150. Konno T, Maeda H, Iwai K, Maki S, Tashiro S, Uchida M, Miyauchi Y. Selective targeting of anti-cancer drug and simultaneous image enhancement in solid tumors by arterially administered lipid contrast medium. *Cancer.* 1984; 54:2367–2374. [PubMed: 6093980]
151. Maki S, Konno T, Maeda H. Image enhancement in computerized tomography for sensitive diagnosis of liver cancer and semiquantitation of tumor selective drug targeting with oily contrast medium. *Cancer.* 1985; 56:751–757. [PubMed: 3160453]

152. Reuveni T, Motiei M, Romman Z, Popovtzer A, Popovtzer R. Targeted gold nanoparticles enable molecular CT imaging of cancer: an in vivo study. *Int. J. Nanomed.* 2011; 6:2859–2864.
153. Hainfeld JF, O'Connor MJ, Dilmanian FA, Slatkin DN, Adams DJ, Smilowitz HM. Micro-CT enables microlocalisation and quantification of Her2-targeted gold nanoparticles within tumour regions. *Br. J. Radiol.* 2011; 84:526–533. [PubMed: 21081567]
154. Chanda N, Kattumuri V, Shukla R, Zambre A, Katti K, Upendran A, Kulkarni RR, Kan P, Fent GM, Casteel SW, Smith CJ, Boote E, Robertson JD, Cutler C, Lever JR, Katti KV, Kannan R. Bombesin functionalized gold nanoparticles show in vitro and in vivo cancer receptor specificity. *Proc. Natl. Acad. Sci.* 2010; 107:8760–8765. [PubMed: 20410458]
155. Kim D, Jeong YY, Jon S. A Drug-Loaded Aptamer–Gold Nanoparticle Bioconjugate for Combined CT Imaging and Therapy of Prostate Cancer. *ACS Nano.* 2010; 4:3689–3696. [PubMed: 20550178]
156. Wang H, Zheng L, Peng C, Guo R, Shen M, Shi X, Zhang G. Computed tomography imaging of cancer cells using acetylated dendrimer-entrapped gold nanoparticles. *Biomaterials.* 2011; 32:2979–2988. [PubMed: 21277019]
157. Popovtzer R, Agrawal A, Kotov NA, Popovtzer A, Balter J, Carey TE, Kopelman R. Targeted Gold Nanoparticles Enable Molecular CT Imaging of Cancer. *Nano Lett.* 2008; 8:4593–4596. [PubMed: 19367807]
158. Kinsella JM, Jimenez RE, Karmali PP, Rush AM, Kotamraju VR, Gianneschi NC, Ruoslahti E, Stupack D, Sailor MJ. X-Ray Computed Tomography Imaging of Breast Cancer by using Targeted Peptide-Labeled Bismuth Sulfide Nanoparticles. *Angew. Chem. Int. Ed.* 2011; 50:12308–12311.
159. Cormode DP, Roessl E, Thran A, Skajaa T, Gordon RE, Schlomka J-P, Fuster V, Fisher EA, Mulder WJM, Proksa R, Fayad ZA. Atherosclerotic Plaque Composition: Analysis with Multicolor CT and Targeted Gold Nanoparticles. *Radiology.* 2010; 256:774–782. [PubMed: 20668118]
160. Cormode DP, Skajaa T, van Schooneveld MM, Koole R, Jarzyna P, Lobatto ME, Calcagno C, Barazza A, Gordon RE, Zanzonico P, Fisher EA, Fayad ZA, Mulder WJM. Nanocrystal Core High-Density Lipoproteins: A Multimodality Contrast Agent Platform. *Nano Lett.* 2008; 8:3715–3723. [PubMed: 18939808]
161. Thakor AS, Gambhir SS. Nanooncology: The future of cancer diagnosis and therapy. *CA. Cancer J. Clin.* 2013; 63:395–418. [PubMed: 24114523]
162. Langereis S, Geelen T, Grull H, Strijkers GJ, Nicolay K. Paramagnetic liposomes for molecular MRI and MRI-guided drug delivery. *NMR Biomed.* 2013; 26:728–744. [PubMed: 23703874]
163. Kaneda MM, Caruthers S, Lanza GM, Wickline SA. Perfluorocarbon Nanoemulsions for Quantitative Molecular Imaging and Targeted Therapeutics. *Ann. Biomed. Eng.* 2009; 37:1922–1933. [PubMed: 19184435]
164. Paulis LEM, Jacobs I, van den Akker NM, Geelen T, Molin DG, E. Starmans LW, Nicolay K, Strijkers GJ. Targeting of ICAM-1 on vascular endothelium under static and shear stress conditions using a liposomal Gd-based MRI contrast agent. *J. Nanobiotechnology.* 2012; 10:1–12. [PubMed: 22221512]
165. Zhang D, Feng X-Y, Henning TD, Wen L, Lu W-Y, Pan H, Wu X, Zou L-G. MR imaging of tumor angiogenesis using sterically stabilized Gd-DTPA liposomes targeted to CD105. *Eur. J. Radiol.* 2009; 70:180–189. [PubMed: 18541399]
166. Mulder WJM, Strijkers GJ, Habets JW, Bleeker EJW, van der Schaft DWJ, Storm G, Koning GA, Griffioen AW, Nicolay K. MR molecular imaging and fluorescence microscopy for identification of activated tumor endothelium using a bimodal lipidic nanoparticle. *FASEB J.* 2005; 19:2008–2010. [PubMed: 16204353]
167. Schmieder AH, Caruthers SD, Zhang H, Williams TA, Robertson JD, Wickline SA, Lanza GM. Three-dimensional MR mapping of angiogenesis with $\alpha 5\beta 1(\alpha \nu \beta 3)$ -targeted theranostic nanoparticles in the MDA-MB-435 xenograft mouse model. *FASEB J.* 2008; 22:4179–4189. [PubMed: 18697838]
168. Boles KS, Schmieder AH, Koch AW, Carano RAD, Wu Y, Caruthers SD, Tong RK, Stawicki S, Hu G, Scott MJ, Zhang H, Reynolds BA, Wickline SA, Lanza GM. MR angiogenesis imaging

with Robo4- vs. $\alpha V\beta 3$ -targeted nanoparticles in a B16/F10 mouse melanoma model. *FASEB J.* 2010; 24:4262–4270. [PubMed: 20585027]

169. Kluza E, Jacobs I, Hectors SJCG, Mayo KH, Griffioen AW, Strijkers GJ, Nicolay K. Dual-targeting of $\alpha v\beta 3$ and galectin-1 improves the specificity of paramagnetic/fluorescent liposomes to tumor endothelium in vivo. *J. Control. Release.* 2012; 158:207–214. [PubMed: 22079810]
170. Kluza E, van der Schaft DWJ, Hautvast PAI, Mulder WJM, Mayo KH, Griffioen AW, Strijkers GJ, Nicolay K. Synergistic Targeting of $\alpha v\beta 3$ Integrin and Galectin-1 with Heteromultivalent Paramagnetic Liposomes for Combined MR Imaging and Treatment of Angiogenesis. *Nano Lett.* 2010; 10:52–58. [PubMed: 19968235]
171. Ghaghada KB, Ravoori M, Sabapathy D, Bankson J, Kundra V, Annapragada A. New Dual Mode Gadolinium Nanoparticle Contrast Agent for Magnetic Resonance Imaging. *PLOS ONE.* 2009; 4:e7628. [PubMed: 19893616]
172. Laurent S, Vander Elst L, Thirifays C, Muller RN. Paramagnetic Liposomes: Inner versus Outer Membrane Relaxivity of DPPC Liposomes Incorporating Lipophilic Gadolinium Complexes. *Langmuir.* 2008; 24:4347–4351. [PubMed: 18338913]
173. Laurent S, Elst LV, Thirifays C, Muller RN. Relaxivities of paramagnetic liposomes: on the importance of the chain type and the length of the amphiphilic complex. *Eur. Biophys. J.* 2008; 37:1007–1014. [PubMed: 18427798]
174. Swanson SD, Kukowska-Latallo JF, Patri AK, Chen C, Ge S, Cao Z, Kotlyar A, East AT, Baker JR. Targeted gadolinium-loaded dendrimer nanoparticles for tumor-specific magnetic resonance contrast enhancement. *Int. J. Nanomed.* 2008; 3:201–210.
175. Baek MJ, Park JY, Xu W, Kattel K, Kim HG, Lee EJ, Patel AK, Lee JJ, Chang Y, Kim TJ, Bae JE, Chae KS, Lee GH. Water-Soluble MnO Nanocolloid for a Molecular T1 MR Imaging: A Facile One-Pot Synthesis, In vivo T1 MR Images, and Account for Relaxivities. *ACS Appl. Mater. Interfaces.* 2010; 2:2949–2955. [PubMed: 20929249]
176. Giraudeau C, Geffroy F, Mériaux S, Boumezeur F, Robert P, Port M, Robic C, Bihan DL, Lethimonnier F, Valette J. 19F molecular MR imaging for detection of brain tumor angiogenesis: in vivo validation using targeted PFOB nanoparticles. *Angiogenesis.* 2012; 16:171–179. [PubMed: 23053783]
177. Southworth R, Kaneda M, Chen J, Zhang L, Zhang H, Yang X, Razavi R, Lanza G, Wickline SA. Renal vascular inflammation induced by Western diet in ApoE-null mice quantified by 19F NMR of VCAM-1 targeted nanobeacons. *Nanomedicine Nanotechnol. Biol. Med.* 2009; 5:359–367.
178. Cassidy MC, Chan HR, Ross BD, Bhattacharya PK, Marcus CM. In vivo magnetic resonance imaging of hyperpolarized silicon particles. *Nat. Nanotechnol.* 2013; 8:363–368. [PubMed: 23644571]
179. Atkins TM, Cassidy MC, Lee M, Ganguly S, Marcus CM, Kauzlarich SM. Synthesis of Long T1 Silicon Nanoparticles for Hyperpolarized 29Si Magnetic Resonance Imaging. *ACS Nano.* 2013; 7:1609–1617. [PubMed: 23350651]
180. Bulte JWM, Kraitchman DL. Iron oxide MR contrast agents for molecular and cellular imaging. *NMR Biomed.* 2004; 17:484–499. [PubMed: 15526347]
181. Jun Y, Seo J, Cheon J. Nanoscaling Laws of Magnetic Nanoparticles and Their Applicabilities in Biomedical Sciences. *Acc. Chem. Res.* 2008; 41:179–189. [PubMed: 18281944]
182. Smolensky ED, Park H-YE, Zhou Y, Rolla GA, Marja ska M, Botta M, Pierre VC. Scaling laws at the nanosize: the effect of particle size and shape on the magnetism and relaxivity of iron oxide nanoparticle contrast agents. *J. Mater. Chem. B.* 2013; 1:2818.
183. Zhang C, Jugold M, Woenne EC, Lammers T, Morgenstern B, Mueller MM, Zentgraf H, Bock M, Eisenhut M, Semmler W, Kiessling F. Specific Targeting of Tumor Angiogenesis by RGD-Conjugated Ultrasmall Superparamagnetic Iron Oxide Particles Using a Clinical 1.5-T Magnetic Resonance Scanner. *Cancer Res.* 2007; 67:1555–1562. [PubMed: 17308094]
184. Hsieh W-J, Liang C-J, Chieh J-J, Wang S-H, Lai I-R, Chen J-H, Chang F-H, Tseng W-K, Yang S-Y, Wu C-C, Chen Y-L. In vivo tumor targeting and imaging with anti-vascular endothelial growth factor antibody-conjugated dextran-coated iron oxide nanoparticles. *Int. J. Nanomedicine.* 2012; 7:2833–2842. [PubMed: 22745546]

185. Castelli DD, Terreno E, Longo D, Aime S. Nanoparticle-based chemical exchange saturation transfer (CEST) agents. *NMR Biomed.* 2013; 26:839–849. [PubMed: 23784956]
186. Opina ACL, Ghaghada KB, Zhao P, Kiefer G, Annapragada A, Sherry AD. TmDOTA-Tetraglycinate Encapsulated Liposomes as pH-Sensitive LipoCEST Agents. *PLOS ONE.* 2011; 6:e27370. [PubMed: 22140438]
187. Vinogradov E, Sherry AD, Lenkinski RE. CEST: From basic principles to applications, challenges and opportunities. *J. Magn. Reson.* 2013; 229:155–172. [PubMed: 23273841]
188. Flament J, Geffroy F, Medina C, Robic C, Mayer J-F, Mériaux S, Valette J, Robert P, Port M, Le Bihan D, Lethimonnier F, Boumezbeur F. In vivo CEST MR imaging of U87 mice brain tumor angiogenesis using targeted LipoCEST contrast agent at 7 T. *Magn. Reson. Med.* 2013; 69:179–187. [PubMed: 22378016]
189. Fayad ZA, Fuster V. Clinical Imaging of the High-Risk or Vulnerable Atherosclerotic Plaque. *Circ. Res.* 2001; 89:305–316. [PubMed: 11509446]
190. Paulis LEM, Jacobs I, van den Akker NM, Geelen T, Molin DG, Starmans LWE, Nicolay K, Strijkers GJ. Targeting of ICAM-1 on vascular endothelium under static and shear stress conditions using a liposomal Gd-based MRI contrast agent. *J. Nanobiotechnology.* 2012; 10:25. [PubMed: 22716048]
191. Smith BR, Heverhagen J, Knopp M, Schmalbrock P, Shapiro J, Shiomi M, Moldovan NI, Ferrari M, Lee SC. Localization to atherosclerotic plaque and biodistribution of biochemically derivatized superparamagnetic iron oxide nanoparticles (SPIONs) contrast particles for magnetic resonance imaging (MRI). *Biomed. Microdevices.* 2012; 9:719–727.
192. Winter PM, Caruthers SD, Zhang H, Williams TA, Wickline SA, Lanza GM. Antiangiogenic Synergism of Integrin-Targeted Fumagillin Nanoparticles and Atorvastatin in Atherosclerosis. *JACC Cardiovasc. Imaging.* 2008; 1:624–634. [PubMed: 19356492]
193. Winter PM, Morawski AM, Caruthers SD, Fuhrhop RW, Zhang H, Williams TA, Allen JS, Lacy EK, Robertson JD, Lanza GM, Wickline SA. Molecular Imaging of Angiogenesis in Early-Stage Atherosclerosis With $\alpha v \beta 3$ -Integrin-Targeted Nanoparticles. *Circulation.* 2003; 108:2270–2274. [PubMed: 14557370]
194. Nahrendorf M, Jaffer FA, Kelly KA, Sosnovik DE, Aikawa E, Libby P, Weissleder R. Noninvasive Vascular Cell Adhesion Molecule-1 Imaging Identifies Inflammatory Activation of Cells in Atherosclerosis. *Circulation.* 2006; 114:1504–1511. [PubMed: 17000904]
195. McAteer MA, Schneider JE, Ali ZA, Warrick N, Bursill CA, von zur Muhlen C, Greaves DR, Neubauer S, Channon KM, Choudhury RP. Magnetic Resonance Imaging of Endothelial Adhesion Molecules in Mouse Atherosclerosis Using Dual-Targeted Microparticles of Iron Oxide. *Arterioscler. Thromb. Vasc. Biol.* 2008; 28:77–83. [PubMed: 17962629]
196. Frias JC, Williams KJ, Fisher EA, Fayad ZA. Recombinant HDL-Like Nanoparticles: A Specific Contrast Agent for MRI of Atherosclerotic Plaques. *J. Am. Chem. Soc.* 2004; 126:16316–16317. [PubMed: 15600321]
197. Christopher R A, Joshua J R, Marina B, Joseph B, Klaus L, Alexander L K. scVEGF Microbubble Ultrasound Contrast Agents: A Novel Probe for Ultrasound Molecular Imaging of Tumor Angiogenesis. *Invest. Radiol.* 2010; 45:579–585. [PubMed: 20733505]
198. Anderson CR, Hu X, Tlaxca J, Declèves A-E, Houghtaling R, Sharma K, Lawrence M, Ferrara K, Rychak JJ. Ultrasound Molecular Imaging of Tumor Angiogenesis with an Integrin Targeted Microbubble Contrast Agent. *Invest. Radiol.* 2011; 46:215–224. [PubMed: 21343825]
199. Ellegala DB, Leong-Poi H, Carpenter JE, Klibanov AL, Kaul S, Shaffrey ME, Sklenar J, Lindner JR. Imaging Tumor Angiogenesis With Contrast Ultrasound and Microbubbles Targeted to $\alpha v \beta 3$. *Circulation.* 2003; 108:336–341. [PubMed: 12835208]
200. Dayton PA, Pearson D, Clark J, Simon S, Schumann PA, Zutshi R, Matsunaga TO, Ferrara KW. Ultrasonic Analysis of Peptide- and Antibody-Targeted Microbubble Contrast Agents for Molecular Imaging of $\alpha v \beta 3$ -Expressing Cells. *Mol. Imaging.* 2004; 3:125–134. [PubMed: 15296677]
201. Willmann JK, Kimura RH, Deshpande N, Lutz AM, Cochran JR, Gambhir SS. Targeted Contrast-Enhanced Ultrasound Imaging of Tumor Angiogenesis with Contrast Microbubbles Conjugated to Integrin-Binding Knottin Peptides. *J. Nucl. Med.* 2010; 51:433–440. [PubMed: 20150258]

202. Leong-Poi H, Christiansen J, Klibanov AL, Kaul S, Lindner JR. Noninvasive Assessment of Angiogenesis by Ultrasound and Microbubbles Targeted to α_v -Integrins. *Circulation*. 2003; 107:455–460. [PubMed: 12551871]
203. Willmann JK, Lutz AM, Paulmurugan R, Patel MR, Chu P, Rosenberg J, Gambhir SS. Dual-targeted Contrast Agent for US Assessment of Tumor Angiogenesis in Vivo. *Radiology*. 2008; 248:936–944. [PubMed: 18710985]
204. Kaufmann BA, Sanders JM, Davis C, Xie A, Aldred P, Sarembock IJ, Lindner JR. Molecular Imaging of Inflammation in Atherosclerosis With Targeted Ultrasound Detection of Vascular Cell Adhesion Molecule-1. *Circulation*. 2007; 116:276–284. [PubMed: 17592078]
205. Yang H, Xiong X, Zhang L, Wu C, Liu Y. Adhesion of bio-functionalized ultrasound microbubbles to endothelial cells by targeting to vascular cell adhesion molecule-1 under shear flow. *Int. J. Nanomedicine*. 2011; 6:2043–2051. [PubMed: 21976979]
206. Demos SM, Alkan-Onyuksel H, Kane BJ, Ramani K, Nagaraj A, Greene R, Klegerman M, McPherson DD. In vivo targeting of acoustically reflective liposomes for intravascular and transvascular ultrasonic enhancement. *J. Am. Coll. Cardiol*. 1999; 33:867–875. [PubMed: 10080492]
207. Hamilton AJ, Huang S-L, Warnick D, Rabbat M, Kane B, Nagaraj A, Klegerman M, McPherson DD. Intravascular ultrasound molecular imaging of atheroma components in vivo. *J. Am. Coll. Cardiol*. 2004; 43:453–460. [PubMed: 15013130]
208. Mulder WJM, Strijkers GJ, Habets JW, Bleeker EJW, van der Schaft DWJ, Storm G, Koning GA, Griffioen AW, Nicolay K. MR molecular imaging and fluorescence microscopy for identification of activated tumor endothelium using a bimodal lipidic nanoparticle. *FASEB J*. 2005; 19:2008–2010. [PubMed: 16204353]
209. Lijowski M, Caruthers S, Hu G, Zhang H, Scott MJ, Williams T, Erpelding T, Schmieder AH, Kiefer G, Gulyas G, Athey PS, Gaffney PJ, Wickline SA, Lanza GM. High-Resolution SPECT-CT/MR Molecular Imaging of Angiogenesis in the Vx2 Model. *Invest. Radiol*. 2009; 44:15–22. [PubMed: 18836386]
210. Luehmann HP, Detering L, Fors BP, Pressly ED, Woodard PK, Randolph GJ, Gropler RJ, Hawker C, Liu Y. PET/CT Imaging of Chemokine Receptors in Inflammatory Atherosclerosis Using Targeted Nanoparticles. *J. Nucl. Med*. 2016; 57:1124–1129. [PubMed: 26795285]
211. Nahrendorf M, Zhang H, Hembrador S, Panizzi P, Sosnovik DE, Aikawa E, Libby P, Swirski FK, Weissleder R. Nanoparticle PET-CT Imaging of Macrophages in Inflammatory Atherosclerosis. *Circulation*. 2008; 117:379–387. [PubMed: 18158358]

Fig. 1. Scanning electron microscopic (SEM) imaging of normal and angiogenic blood vessels. (a) SEM image of polymer cast of normal microvasculature (vasa vasorum of rat carotid sinus). (b) SEM image of polymer cast of tumor microvasculature (xenograft of human tumor in nude mouse). **Reproduced with permission from reference [10].**

Fig. 2.

Illustration shows a comparison between healthy and tumor vasculature. Differences between (a) normal and (b) tumor vasculature in terms of structure and function with regions of severe hypoxia indicated by blue shading. BM, basement membrane; EC endothelial cell; IFP, interstitial fluid pressure. **Reproduced with permission from reference [29].**

Fig. 3.
Baseline endothelial shear stress patterns along the course of a coronary artery obstruction.
Reproduced with permission from reference [32].

Fig. 4. Effect of the geometry of nanoparticles on their (a) margination and (b) binding avidity. Shape, ligand length, and polymer flexibility all play a role in the active fractional area of a nanoparticle (AFAC). For a sphere, the AFAC is defined as $(L - d_b)/D_c$, where L is the length of the ligand, d_b is the binding distance between the nanoparticle and the receptor, and D_c is the diameter of the nanoparticle. **Reproduced with permission from reference [35].**

Fig. 5.

Detection of micrometastasis using nuclear imaging and an $\alpha_v\beta_3$ integrin-targeting gold nanoparticle (AuNP) radiotracer labeled with ^{99m}Tc . (a) Representative coronal image using whole-body planar gamma scintigraphy shows the detection of micrometastatic sites in the lungs in a mouse bearing 4T1 breast cancer metastasis at 30 min after injection of the AuNP radiotracer (Gamma Medica FLEX small animal-imaging instrument, X-SPECT system). The animal was injected with a dose of 50 μCi of the ^{99m}Tc -AuNP radiotracer. (b) The gamma scintigraphy image was coregistered with a 99- μm -resolution micromorphological image obtained by using a Siemens Inveon micro-CT and a liposome-based iodinated contrast agent (yellow arrows indicate the location of metastatic sites). (c) At the end of the nuclear imaging session, the lungs of the animals were perfused, excised and imaged ex vivo using the Gamma Medica X-SPECT system and a CRi Maestro fluorescence imaging system, indicating the colocalization of the AuNP radiotracer and 4T1 metastatic cells expressing GFP. (d) Representative coronal image using planar gamma scintigraphy shows a healthy mouse 30 min after injection of the AuNP radiotracer. **Reproduced with permission from reference [61].**

Fig. 6.

Multimodal *in vivo* imaging of vasculature, vascular permeability, integrin expression, and blood flow in an orthotopic 4T1 mammary tumor in mouse. (a) The same animal was imaged with contrast enhanced micro-CT using an iodinated nanoparticle, Fluorescence Molecular Tomography (after injection of integrin-targeting probe) and standard perfusion CT. The high-resolution angiogram (28 μ m resolution) was co-registered with the images of vascular permeability, integrin expression (FMT imaging), and tumor blood flow (perfusion CT). **Reproduced with permission from reference [44].**

Fig. 7.

MR imaging of atherosclerotic lesions using a VCAM-1-targeted magnetofluorescent nanoparticle based on iron oxide. (a) Using contrast enhanced MRI, angiography was performed on cholesterol-fed apoE^{-/-} mice to identify vascular abnormalities. Images were then obtained before (b) and 24 hours after (c) injection of the targeted nanoparticle. (d) Ex vivo MRI confirms extensive low signal changes in the aortic wall induced by nanoparticle accumulation, further validated by epifluorescence imaging of the Cy 5.5 co-label on the nanoparticle. (e-h). Histological validation of MR findings. **Reproduced with permission from reference [116].**

Table 1

Characteristics of molecular imaging modalities.

Modality	Spatial resolution	Penetration depth	Sensitivity (mol/L)	Cost
PET	1–2 mm	no limit	High (10^{-11} – 10^{-12})	High
SPECT	1–2 mm	no limit	High (10^{-10} – 10^{-11})	Medium
CT	50–200 μm	no limit	Low (10^{-1} – 10^{-4})	Low
MR	25–100 μm	no limit	Low (10^{-3} – 10^{-5})	High
Fluorescence	2–3 mm	<1 cm	High (10^{-9} – 10^{-12})	Low
US	50–500 μm	mm-cm	Medium	Low