

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart
Association®



Learn and Live SM

Molecular Imaging in Atherosclerosis, Thrombosis, and Vascular Inflammation

Robin P. Choudhury and Edward A. Fisher

Arterioscler. Thromb. Vasc. Biol. 2009;29:983-991; originally published online Feb 12, 2009;

DOI: 10.1161/ATVBAHA.108.165498

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association,
7272 Greenville Avenue, Dallas, TX 75214

Copyright © 2009 American Heart Association. All rights reserved. Print ISSN: 1079-5642. Online
ISSN: 1524-4636

The online version of this article, along with updated information and services, is
located on the World Wide Web at:

<http://atvb.ahajournals.org/cgi/content/full/29/7/983>

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular
Biology is online at

<http://atvb.ahajournals.org/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters
Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax:
410-528-8550. E-mail:

journalpermissions@lww.com

Reprints: Information about reprints can be found online at

<http://www.lww.com/reprints>

Molecular Imaging in Atherosclerosis, Thrombosis, and Vascular Inflammation

Robin P. Choudhury, Edward A. Fisher

Abstract—Appreciation of the molecular and cellular processes of atherosclerosis, thrombosis, and vascular inflammation has identified new targets for imaging. The common goals of molecular imaging approaches are to accelerate and refine diagnosis, provide insights that reveal disease diversity, guide specific therapies, and monitor the effects of those therapies. Here we undertake a comparative analysis of imaging modalities that have been used in this disease area. We consider the elements of contrast agents, emphasizing how an understanding of the biology of atherosclerosis and its complications can inform optimal design. We address the potential and limitations of current contrast approaches in respect of translation to clinically usable agents and speculate on future applications. (*Arterioscler Thromb Vasc Biol.* 2009;29:983-991.)

Key Words: atherosclerosis ■ inflammation ■ molecular imaging

Appreciation of the molecular and cellular processes of atherosclerosis, thrombosis, and vascular inflammation opens the way for commensurately sophisticated approaches to disease characterization through imaging.¹⁻⁶ The common goals of molecular imaging approaches are to accelerate and refine diagnosis, provide insights that reveal disease diversity, guide specific therapies, and monitor the effects of those therapies. To these ends, a range of contrast methodologies are in development across a number of modalities. This review will undertake a comparative analysis of imaging modalities applicable to atherosclerosis, thrombosis, and vascular inflammation and highlight some of the molecular, cellular, and functional targets that show greatest potential. It will compare the attributes of different approaches and relate these to specific applications, emphasizing the opportunities and challenges for each. Other reviews in this series will provide detailed systematic consideration of individual modalities.

As the repertoire of molecular contrast agents expands and more show potential in proof of principle studies, we will consider the routes and obstacles to the development of agents that might be used in the clinical setting.

Targets

Vascular disease is relatively privileged compared, for instance, to neurological imaging because many of the targets are accessible to the blood and blood-borne reagents. There are also specific impediments. Blood vessels are often deeply

located structures, which can restrict the application of low penetrance techniques, such as fluorescence imaging or ultrasound unless intravascular imaging systems are developed. In addition, atherosclerotic plaques are relatively small structures and there are challenges of movement because of cardiac and respiratory motion. High shear stresses of blood in large arteries can be challenging to particulate contrast agents.

To highlight potential imaging targets, it may be helpful to consider atherogenesis in terms of (1) early processes; (2) progression to more advanced lesions; and (3) thrombotic complications. Specific imaging targets, discussed below, are highlighted in Figure 1).

Events in Early Atherogenesis

Early in atherogenesis, disordered endothelial function accelerates the deposition of apolipoprotein B-containing lipoprotein particles in the subendothelial space.⁷ A fraction of these particles are retained, which promotes local inflammation, characterized by the release of soluble signaling factors, including chemokines,⁸ and by the expression of endothelial cell adhesion molecules, eg, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and P-selectin,⁹⁻¹¹ which recruit mononuclear leukocytes, especially monocytes and T-lymphocytes, to the arterial wall.¹² Recruited monocytes differentiate into macrophages and upregulate several scavenger receptors capable of binding modified forms of low density lipoproteins (LDL) including scavenger receptor types AI and AII (SR-AI, SRAII),

Received December 31, 2008; revision accepted February 3, 2009.

From the Department of Cardiovascular Medicine (R.P.C.), University of Oxford, UK; and New York University School of Medicine (E.A.F.), New York.

Correspondence to Dr Robin Choudhury, Department of Cardiovascular Medicine, John Radcliffe Hospital, Oxford. OX3 9DU, UK. E-mail robin.choudhury@cardiov.ox.ac.uk

© 2009 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol is available at <http://atvb.ahajournals.org>

DOI: 10.1161/ATVBAHA.108.165498

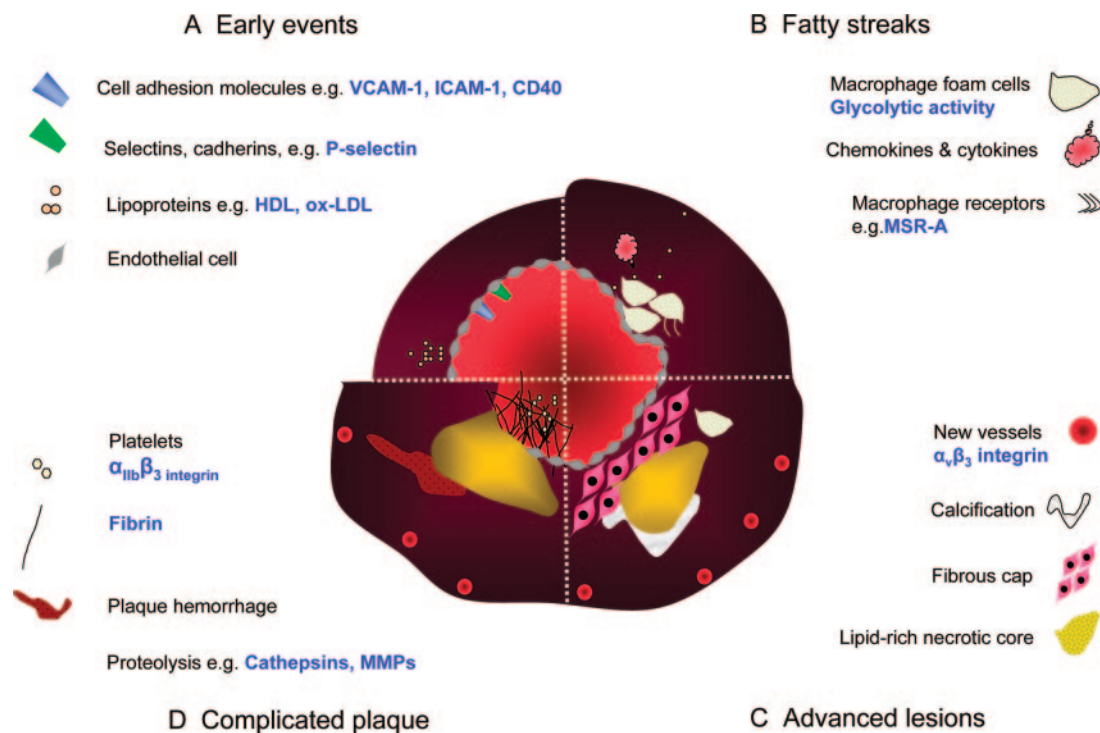


Figure 1. Targets for molecular and cellular imaging in atherosclerosis, thrombosis, and vascular inflammation. Schematic cross section through the wall of a large artery indicating the progression of atherosclerosis (clockwise from top left) and the accumulation of specific molecular imaging targets (for details, please refer to text). For a given pathological process, imaging targets are listed in blue.

CD36, CD68, LOX-1, and SR-PSOX/CXCL16.¹³ Modified LDL taken up via scavenger receptors is delivered to lysosomes, where enzymes hydrolyze cholesteryl esters to free cholesterol and fatty acids. Inside macrophages, the enzyme acyl-CoA:cholesterol acyltransferase (ACAT) catalyzes the formation of cholesteryl ester, which accumulates in characteristic foamy deposits.

Progression of Atherosclerosis

The net rate of accumulation of cholesterol in the plaque reflects the difference between its rate of deposition and extraction from the plaque by high density lipoproteins. High local concentrations of cholesterol may be associated with apoptosis and necrosis of plaque macrophages.¹⁴ When intracellular storage capacity is exceeded, free cholesterol is able to accumulate in the extracellular domain. Ruptured plaques, which underlie acute atherothrombotic events, commonly contain high concentrations of macrophages, particularly in the superficial “shoulder” region.^{15,16} Plaque macrophages secrete matrix metalloproteinases that digest the stabilizing connective tissue elements of plaque promoting vulnerability to rupture.^{17,18} In addition to destabilizing plaque, macrophages also secrete prothrombotic tissue factor, which accelerates thrombus formation after rupture or erosion.¹⁹ However, because MMP activity is regulated (by tissue inhibitors of metalloproteinases or TIMPs), it may not be sufficient to image the mere presence of MMPs. More meaningful insights will be derived from assessment of the proteolytic activity of MMPs within the plaque, analogous to *in situ* zymography.¹⁸

Thrombotic Complications

Rupture or erosion of an atherosclerotic plaque is associated with local deposition of activated platelets, fibrin, and tissue

factor. The ability to localize, quantify, and characterize acute thrombus rapidly and noninvasively would be of great clinical utility in acute vascular syndromes and could inform the most appropriate choice of therapy.

Generic Requirements for Molecular Imaging

Conceptually, molecular imaging requires multiple functional components, though in practice more than one function can be integrated into a single element. Localization and retention at the site of interest is typically via (1) a ligand with both affinity and specificity for the target (eg, antibody, antibody fragment, peptide, aptamer or oligosaccharide). A contrast or (2) signaling element (eg, iron oxides, gadolinium, radionuclides, fluorochromes, gas-filled microbubbles) may be incorporated directly into the ligand or may be conveyed by a (3) carrier vehicle (eg, micelle, perfluorocarbon nanoparticle, synthetic lipoprotein particle, polymer-derived microparticle, carbon nanotube, Figure 2). Determinants of accessibility and mechanisms of conveyance to the target (eg, diffusion, mass flow, receptor-mediated internalization, pinocytosis, intracellular carriage) will depend on the (4) physical and chemical properties the contrast agent (eg, size, charge, hydrophobic/hydrophilic exterior, specific surface molecules). Design-for-purpose of a contrast agent or class of agents requires consideration and optimization of these multiple elements.

Ligand

Antibodies

A ligand should possess both specificity and affinity for the target of interest. In experimental studies, antibodies have

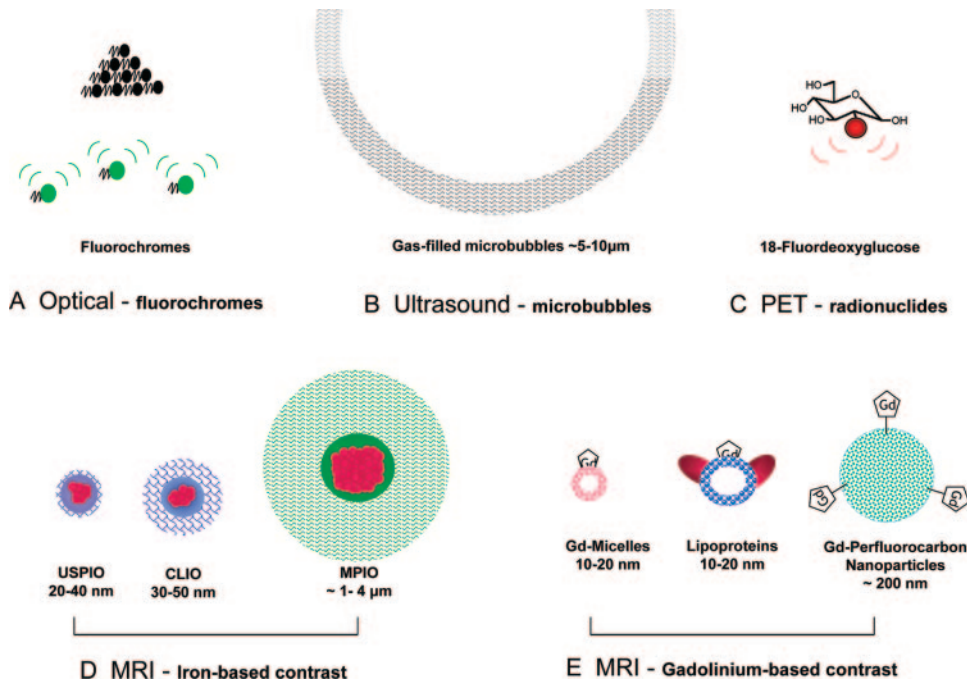


Figure 2. Contrast agent vehicles for molecular imaging in atherosclerosis, thrombosis and vascular inflammation (not to scale). A, Fluorochromes comprise small molecules that may be delivered in quenched form with fluorescence potential only liberated after specific enzymatic cleavage. B, Acoustically active microbubbles are typically relatively large (5 to 10 μm diameter) structures made from albumin or lipids. Bound to activated endothelium, even single bubbles can be detected. C, PET detects, with high sensitivity, positron emission from radionuclides, commonly ^{18}F fluorine. Small molecules, including drugs, can be adapted to incorporate suitable radionuclides enabling both localization and quantification of molecular expression. Magnetic resonance molecular imaging relies on the delivery of relatively high payloads of either (D) iron oxides or (E)

gadolinium chelates. Iron oxide particles (size range $\approx 10\text{ nm}$ to $5\ \mu\text{m}$) are typically contained within polymer shells. Gadolinium contrast requires interaction with local water molecules, and the chelates therefore decorate the surface of the carriage vehicle.

been used against numerous targets that include VCAM-1,²⁰ P-selectin,^{21,22} ICAM-1,^{23,24} $\alpha_v\beta_3$ integrin,²⁵ epitopes on oxidized LDL,^{26,27} and macrophage scavenger receptor.²⁸ Thus antibodies have proven effective despite potential steric limitations imposed by their relatively large size. Potential immunogenicity can be attenuated by the use of modified or “humanized” antibodies²⁹ and by the use of single chain antibodies.^{30,31} However, antibody production is also relatively complex and expensive to generate in a quantity that would be required for use as a clinical reagent. Alternative approaches have used peptides, such as the RGD motif that binds to integrins,³² and oligosaccharides.^{33–35} In a recent novel approach, synthetic structures, incorporating proteins with posttranslational modifications, have been generated to achieve functional mimicry of naturally occurring ligands, eg, P-selectin glycoprotein ligand, and have targeted vascular inflammation.³⁶

Multiple Ligands

In large and medium arteries that are affected by atherosclerosis, high shear stresses can impair receptor-ligand interactions. Low-affinity interactions are potentially enhanced by polyvalent ligand presentation, which may be accomplished by conjugating the ligand of interest to microparticles.³⁷ Physiologically, leukocytes are arrested at sites of inflammation by using multiple receptor ligand interactions. Using combinations of ligand pairings (eg, antibodies against VCAM-1 and P-selectin²² or antibody to ICAM-1 and sialyl-Lewis X, targeting selectins³⁸) on the surface the same particles, binding can be enhanced by up to an order of magnitude compared to binding mediated by single interaction binding. Retention at target can be further amplified by strategies that result in local accumulation. For example, a

high-specificity probe targeting VCAM-1 and internalized by endothelial cells after binding has been developed through phage display.³⁹ In this way, ligand characteristics can be used to enhance sensitivity for imaging the endothelial monolayer, a cell-type that is of crucial importance but low abundance compared to other cell types of the arterial wall.

Phage Display

Phage display is a powerful, if labor-intensive, technique that allows a huge range of potentially useful diagnostic and therapeutic antibodies to be evaluated in a functionally relevant context—and for the useful ones to be produced in large quantities. Bacteria and bacterial viruses, known as phage, are used to produce synthetic antibodies with highly diverse target recognition (“variable”) regions based on the corresponding DNA (cDNA) obtained from reverse transcription of mammalian B-lymphocyte total messenger RNA (mRNA). The phage are engineered so that the antibody is fused to a protein that is expressed on the phage coat and the gene encoding for that specific antibody is contained within the coat. Collections of the antibody-coated phage (typically in the order of 10^9 separate permutations) constitute a phage library. Those of interest can be selected for by solid phase immunosorption mediated by the surface antibody and amplified once isolated. In this way, further ligands have been identified that target cell adhesion molecules^{39–41} and activated platelets via the activate conformation of glycoprotein IIb/IIIa (Figure 3).^{30,31,42,43} In another variation of phage display, instead of antibodies, peptides are expressed on the phage coat, and those that interact with receptors or other surface molecules on the target cells can be similarly identified and enriched.³⁹

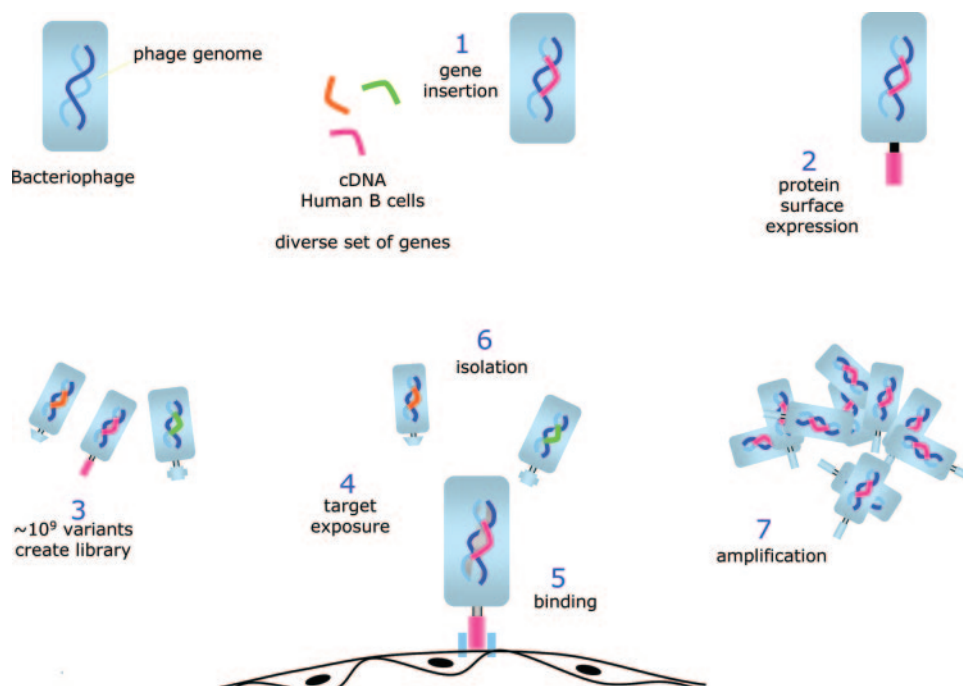


Figure 3. Phage display is a powerful strategy for the identification and production of ligands that target specific molecules or particular biological states, even without knowledge of the target molecule. Bacteriophage are fused with diverse genes, generated, for example, from human B cells. Incorporated cDNA from the diversity source generates the protein product, which the phage expresses on its surface. Exposure of the phage library to the target of interest, eg, activated vascular endothelium, allows active ligands to be isolated and subsequently amplified to yield workable quantities for the construction of imaging probes.

Kinetics

The on-off kinetics of ligand binding are important. Clinically useful reagents are likely to display relatively rapid “on” kinetics that allow imaging soon (minutes to hours) after contrast administration. Although this may be feasible for imaging intravascular targets such as endothelial adhesion molecules or components of thrombus, delivery to targets within the plaque is likely to take longer (hours to days). To enable sequential imaging in determining disease activity and response to treatment, swift removal and clearance of the contrast agent are desirable characteristics. Most molecular contrast agent studies to date report single time point imaging that provides limited insight into the “on-kinetics” and often no evaluation of tissue residency, redistribution, or disposal. If data are to be useful in quantification of the target of interest, the kinetics need to be reproducible and predictable.

Contrast or Signaling Agent

Optical Imaging

Molecular imaging modalities vary intrinsically in their sensitivities. Fluorescence techniques possess high sensitivity, but are limited by tissue penetrance, including in blood. Fluorescent probes that can be incorporated into relatively small molecules that possess, or retain, biological function bring accessibility, combined with a functional versatility that may be lacking where larger carrier vehicles are required (eg, micelles, liposomes, lipoproteins; see below). Use of multiple fluorochromes targeting different molecules opens the way for multiplex imaging to provide insights of greater complexity. Besides reporting simple accumulation, polymeric probes can be constructed in which fixed close proximity of fluorochromes limit their capacity for excitation, such that fluorescence is quenched before specific enzymic cleavage that liberates the fluorescent capability. In this way a level of functional specificity is generated. Examples in vascular

imaging include mapping the activity of the macrophage-associated enzymes cathepsins B and K in mouse atherosclerosis^{44,45} and matrix metalloproteinases.⁴⁶ The precise depth of tissue penetrance varies with the fluorochrome and the tissue under evaluation. Although quantitative depth-resolved NIR fluorescence systems are routinely available for mice,⁴⁷ there are limitations in the assessment of deep structures such as the coronary arteries in humans. As a result, this type of imaging is likely to make its greatest contributions as an experimental tool. Development of catheter-mounted fiberoptic devices for use in intraarterial imaging offers promise for clinical application, particularly if this can be combined with a mode of intravascular anatomic imaging, such as intravascular ultrasound, optical coherence tomography, or MRI.^{48–52}

PET and SPECT

Radionuclides provide a high degree of sensitivity, with detection possible in the picomolar range. Single positron emission tomography (SPECT) has been used to image apoptosis, by targeting annexin A5 in rabbits⁵³ and to track monocytes in experimental mouse atherosclerosis.⁵⁴ To date, ¹⁸Fluoro-deoxyglucose (¹⁸FDG) has been most commonly used PET agent in vascular imaging.^{55–57} This modified glucose analogue is transported into cells that are active in glycolysis. However, inability to pass through the glycolytic pathway leads to intracellular accumulation. Imaging with ¹⁸FDG has shown initial promise in the aorta and carotid arteries, where its accumulation has been shown to correlate with macrophage infiltration, determined by immunohistochemistry in explanted carotid endarterectomy samples.⁵⁸ and to change in response to treatment.⁵⁹ Proximity to the highly metabolically active myocardium, and consequent signal spillover, limits use of ¹⁸FDG for coronary imaging. However, PET imaging is not limited to ¹⁸FDG, and there is an expanding range of radio-ligands.

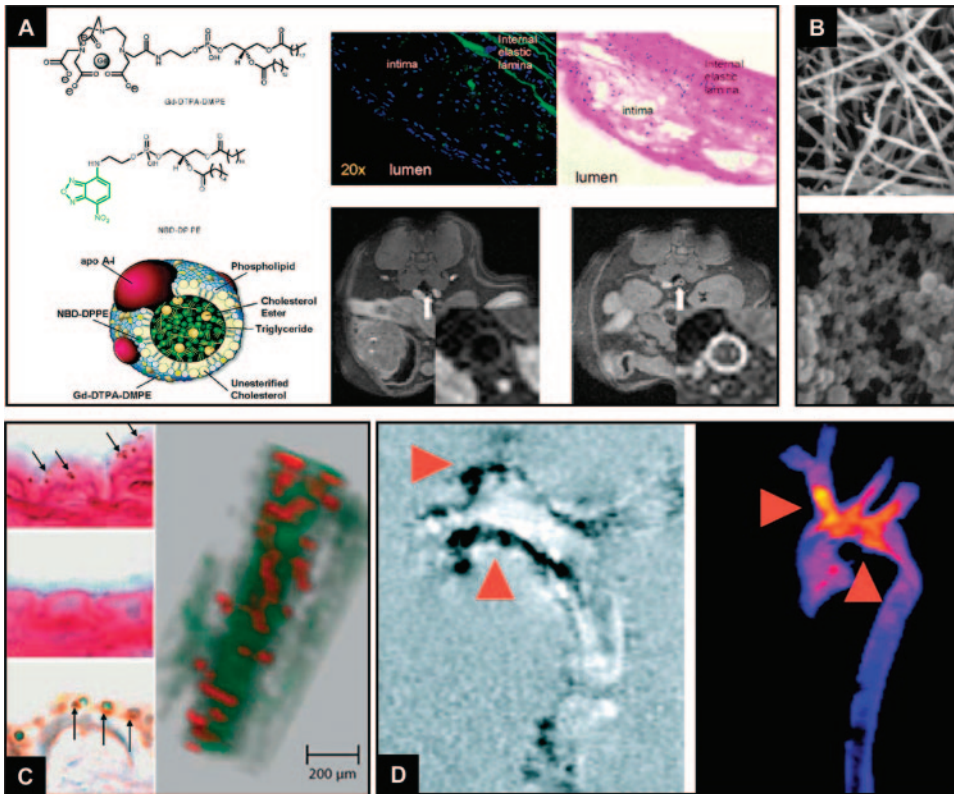


Figure 4. Imaging in atherosclerosis (A), thrombosis (B and C), and vascular inflammation (D). A, Reconstituted high-density lipoprotein particles containing both fluorochrome and a gadolinium chelate (left) can be used for bimodal imaging of atherosclerosis. After intravenous injection the particles accumulate in the plaques of apoE^{-/-} mice and are seen as green punctate structures with fluorescence imaging. T1-weighted MRI shows marked concentric contrast enhancement 24 hours after injection compared to the pre-contrast appearances (bottom right).⁷⁹ B, Scanning electron micrograph demonstrating fibrin filaments (upper panel). Binding of Gd-rich nanoparticles (lower panel) to the surface of the thrombus results in contrast enhancement on T1-weighted MRI (not shown).⁹⁰ C, Microparticles of iron oxide bearing a ligand recognizing the activated form of glycoprotein IIb/IIIa are shown bound to freshly denuded mouse femoral artery. In the lower left panel, P-selectin staining reveals a car-

pet of adherent platelets colocalized with spherical microparticles. Semiautomated segmentation highlights the distribution of MPIO binding along the injured arterial segment.³¹ D, Peptides generated by phage display recognize VCAM-1 expressed in activated endothelium and can be converted to bimodal contrast agents by their conjugation to iron nanoparticles and a fluorescent reporter. Injected into apoE^{-/-} mice, these specific probes accumulate within endothelial cells at sites that develop atherosclerosis appearing intensely dark on ex vivo MRI (left) confirmed by fluorescence imaging (right).⁴⁰

A further appeal of this modality is the opportunity to radiolabel drugs and to quantify receptor binding and pharmacodynamics in vivo. The lack of anatomic detail from PET images, can be largely addressed by coregistration with either CT or MRI.

Of approaches to molecular imaging, PET and SPECT are the only ones that are established in clinical use, though there are also important logistical drawbacks. As radioisotopes, the contrast reagents are expensive and are not stable for more than short periods. Furthermore access is relatively limited both to PET scanners and radionuclide sources.

Magnetic Resonance Imaging

By comparison, MRI provides a high degree of spatial resolution and soft tissue contrast yielding excellent anatomic detail, but is of inherently low sensitivity for molecular imaging applications. In MRI, appearances are determined by the density and local environment of water protons. MR contrast agents exert indirect effects by altering the properties of these protons and their local environment. Compared to fluorescence or PET imaging, attainment of contrast in MRI requires the delivery of relatively large quantities of contrast agent to the site of interest (micromolar range). Typically, these agents comprise gadolinium ions chelated to small molecules (to attenuate toxicity of gadolinium ions). Gadolinium shortens T1, leading to bright appearance or positive contrast on T1-weighted images.⁶⁰ Alternatively, superpara-

magnetic iron oxides confer greater sensitivity. These contrast agents induce proton dephasing, usually manifest as intensely negative contrast on T2*-weighted images. The range of iron-based contrast agents and methods for obtaining “positive contrast” effects have recently been reviewed comprehensively.^{61,62} Importantly, for enhancing sensitivity in molecular imaging applications, the contrast effects derived from iron oxides can manifest in a volume that is orders of magnitude greater than the physical size of the particles that contain them.^{63,64} Shapiro et al have shown how micron-size-range (0.9 to 8.5 μm) particles containing iron oxide can exert contrast effects that extend ≈ 50 times the physical dimension of the particle. Initially used in cellular imaging applications, microparticles have more recently been applied to molecular imaging in vascular inflammation,⁶⁵ atherosclerosis,²² and platelet thrombosis (Figure 4).^{31,42} Further consideration of the different sizes and composition of iron oxide-containing particles is given below.

Analogous to the enzymatic cleavage sites incorporated into quenched-state fluorochromes, sophisticated MR agents have been engineered to conceal gadolinium from tissue water (thus quenching its contrast efficacy), unless a specific enzyme-mediated cleavage occurs. In this way, it has been possible to image β -galactosidase activity as a marker of transgene expression.⁶⁶ Similarly, activity of the enzyme myeloperoxidase (released from activated neutrophils and macrophages, and elevated in blood in acute coronary syn-

dromes) can be detected using a conditionally activated probe in which exposure to MPO causes oligomerisation of gadolinium-rich generating particles with higher T1 relaxivity than the monomeric source compound.⁶⁷

Ultrasound

Ultrasound provides relatively low resolution anatomic data, but with the very great advantages of being widely available, cheap, and portable. Numerous investigators have adopted approaches in which molecular imaging has been attained through the use of targeted gas-filled micobubbles.^{68–71} These particles, such as liposomes⁷² or gas-filled phospholipid micobubbles,⁶⁹ produce intense acoustic reflection. By this approach it has been possible to image a range of targets similar to those described for MRI (above). Specifically, ICAM-1,⁷² VCAM-1,⁷³ P-selectin,²¹ fibrin,⁷² and integrins⁶⁸ have all been imaged with targeted ultrasound probes.

Carriers

For the high sensitivity modalities, eg, PET and fluorescence imaging, the contrast agent may simply comprise a small molecule. For lower sensitivity techniques eg, ultrasound and MRI, a carrier vehicle or amplification particle (eg, liposome, micelle, lipoprotein, carbon nanotube, dextran particle) may be required.

MRI

Nanometer-size-range iron oxide particles (ultrasmall particles of iron oxide, USPIO) can be used for cellular imaging purposes and have been used as passive contrast agents to identify plaque macrophages in humans.^{74,75} To provide a magnetic “shield” that minimizes aggregation, these particles have a polymeric coat surrounding a nucleus of iron oxides. Commonly the glucose-based polymer dextran is used, because it is relatively straightforward to manipulate and is biocompatible, including for use in humans. To add structural stability, cross-linked preparations (cross linked iron oxides, termed “CLIO”) have been developed with functionalized exteriors that permit covalent conjugation of surface ligands for molecular imaging purposes.⁷⁶

USPIO have a long half-life in the blood, which is a positive attribute for applications such as the measurement tissue perfusion. However, this property is more of a hindrance in targeted contrast agents because it leads to high background contrast for an extended period. A further potential drawback of USPIO is that contrast is manifest in T2*-weighted images as indistinct areas of low signal that can be difficult to distinguish from the ordinary heterogeneity of normal tissue.⁷⁷ Furthermore, because USPIO can be taken up nonspecifically and can extravasate passively, particularly in the leaky vessels associated with sites of inflammation, there is potential to compromise the specificity of molecular targeting.⁷⁸ We have used much larger microparticles of iron oxide (0.9 to 4.5 μm ; “MPIO”) for molecular imaging applications. The payload of iron is high and the contrast effect is pronounced. For imaging endovascular structures, MPIO possess several positive attributes. First, the particles convey a payload of iron that is many orders of magnitude greater than USPIO. Second, the effects of MPIO on local

magnetic field homogeneity, and therefore detectable contrast, extend for a distance ≈ 50 times the physical diameter of the microparticle.⁶³ Third, unlike USPIO, the relatively large size of MPIO means that they are less susceptible to extravasation or nonspecific uptake by endothelial cells⁷⁸ and therefore retain specificity for molecular targets expressed on the vascular endothelium. However, the corollary of this is an inability of MPIO to image epitopes found deeper within the plaque.

The ability to manipulate the physical properties and composition of contrast particles presents opportunities to harness physiological pathways. For instance, we have isolated and delipidated normal human HDL to obtain its apolipoproteins, mainly apolipoprotein (apo) A-I. The apolipoproteins can then be extracted and reconstituted with phospholipids, with or without unesterified cholesterol. To generate a MR contrast agent, phospholipid-based Gd-DTPA-DMPE is incorporated into the reconstituted particle of approximately 9 nm diameter. For confocal fluorescence microscopy studies, a fluorescent phospholipid with a green emission was also added. More recently, in some preparations, the Gd-DTPA-DMPE and fluorescent phospholipid have been added to intact HDL by gentle sonication. In either type of reconstitution, when intravenously injected, these small particles accumulate within atheromatous plaques, following HDL pathways into plaque macrophages (Figure 4).⁷⁹ To avoid the requirement for human blood-derived HDL, we have developed an apoA-I-mimicking peptide, 37pA, to replace native apoA-I, thereby avoiding the lengthy separation procedure required to isolate HDL. Particles formed from 37pA have been reported to perform functions of native HDL, such as cholesterol efflux and binding to the ABCA-1 transporter and are effective imaging agents.⁸⁰

Ultrasound

Gas-filled micobubbles for ultrasound imaging comprise albumin, lipid, or perfluorocarbon shells around a gas-filled core, (typical size range 5 to 20 μm). The sensitivity of ultrasound is such that single static micobubbles can be detected with transcutaneous ultrasound. Furthermore, bubbles can be instantaneously destroyed by delivery of a sonic pulse, providing a way to confirm binding, an opportunity to interrogate the dynamics of binding and a method for local delivery of any encapsulated drugs.

Clearance

Anticipating potential clinical application, contrast clearance is an important consideration. In the first place, rapid blood phase clearance may be required to remove background signal to permit identification of retained contrast at the site of interest. Afterward, clearance from the target site will permit repeated measures, for example to monitor response to treatment. In the final phase, the agent should undergo disposal or excretion, with or without prior dismantling to component elements. For example it has been proposed that microparticulate contrast agents might be dismantled in the reticuloendothelial system with iron incorporation into the general pool. Variations in size and surface composition can influence routes and rates of disposal.^{81,82} To date, though,

studies that have focused on proof of principle imaging have typically neglected detailed evaluation of effects of variable dosing, timing, and routes of disposal.

Toxicity

Clearly, a favorable safety profile is a prerequisite for clinical use, and the regulatory requirements for demonstration of safety are no less stringent for diagnostic contrast agents than for therapeutic agents. Areas requiring evaluation include immunogenicity, radioactivity, chemical toxicity, potential for pharmacological action, physical toxicity (eg, vessel plugging), potential for accumulation on repeated dosing.

In general little is known of these, and studies tend to be small scale, organ- or system-specific with a lack of dose ranging evaluation, effect of repeat administrations, time course, or anything other than immediate toxicology. As candidates for translation to human emerge, evaluation of these parameters will be vital.

Therapeutic Options

Delivery of targeted agents that can be localized to areas of disease and quantified presents clear opportunities for efficient delivery of site-specific therapies.^{83,84} In vascular disease, these might include agents that target endothelial function, inflammation, angiogenesis, cellular proliferation, and coagulation.

Reduced neointimal formation in a rabbit model of in-stent restenosis has been accomplished using systemically delivered albumin-nanoparticles containing paclitaxel.⁸⁵ Recently reported was the use of nanoparticle delivery of prednisolone, encapsulated in liposomes targeting chondroitin sulfate proteoglycans in the vessel wall, which are exposed after stent implantation.⁸⁶ A similar approach has incorporated the antiproliferative agents doxorubicin and paclitaxel in to nanoparticles targeting tissue factor. Coloaded with gadolinium, these particles showed demonstrable T1 effects with MR at 4.7T.⁸⁷ Moreover, by using ¹⁹F incorporated into perfluorocarbon nanoparticles, these authors propose a method for direct spectroscopic quantification of particle accumulation. An elegant approach to drug delivery using imaging particles has involved incorporation of the lipid soluble antiangiogenic compound fumagallin into nanoparticles targeted to $\alpha_v\beta_{III}$ integrin, expressed in the neovasculature of rabbit atherosclerosis.⁸⁸ Future iterations of combined therapeutic/diagnostic agents may benefit from advances in polymer science that will enable delivery of biologically inert particles that are spontaneously degraded without deleterious effects.⁸⁹

The Future and Clinical Application

Future technical developments will focus on each of the components identified above with specific interest in the development of more sophisticated targeting strategies and the use of multimodal imaging probes that will combine sensitivities to make best use of integrated MRI and PET. Already, there has been much progress in the area of molecular imaging for vascular diseases. Yet the hurdles to translation into the clinical realm are considerable. Although ultrasound and MRI molecular contrast agents have been used in humans, of the modalities discussed above, only PET

and SPECT have molecular contrast agents in routine clinical use. A challenge to scientists and their commercial partners is how to pick the right agent at the right time in its development and to define and application to move forward to clinic.

Sources of Funding

Dr Choudhury's laboratory is funded by the Wellcome Trust, the British Heart Foundation, and by the Oxford Comprehensive Biomedical Research Centre, NIHR funding scheme. Dr Fisher's work in this area is funded by NIH grant R01HL078667.

Disclosures

None.

References

1. Choudhury RP, Fuster V, Badimon JJ, Fisher EA, Fayad ZA. MRI and characterization of atherosclerotic plaque: emerging applications and molecular imaging. *Arterioscler Thromb Vasc Biol.* 2002;22:1065–1074.
2. Choudhury RP, Fuster V, Fayad ZA. Molecular, cellular and functional imaging of atherothrombosis. *Nat Rev Drug Discov.* 2004;3:913–925.
3. Jaffer FA, Libby P, Weissleder R. Molecular and cellular imaging of atherosclerosis: emerging applications. *J Am Coll Cardiol.* 2006;47:1328.
4. Jaffer FA, Libby P, Weissleder R. Molecular imaging of cardiovascular disease. *Circulation.* 2007;116:1052–1061.
5. Lindsay AC, Choudhury RP. Form to function: current and future roles for atherosclerosis imaging in drug development. *Nat Rev Drug Discov.* 2008;7:517–529.
6. Lipinski MJ, Fuster V, Fisher EA, Fayad ZA. Technology insight: targeting of biological molecules for evaluation of high-risk atherosclerotic plaques with magnetic resonance imaging. *Nat Clin Pract Cardiovasc Med.* 2004;1:48–55.
7. Williams KJ, Tabas I. The response-to-retention hypothesis of early atherogenesis. *Arterioscler Thromb Vasc Biol.* 1995;15:551–561.
8. Gerszten RE, Garcia-Zepeda EA, Lim YC, Yoshida M, Ding HA, Gimbrone MA Jr, Luster AD, Luscinskas FW, Rosenzweig A. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. *Nature.* 1999;398:718–723.
9. Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. *Arterioscler Thromb Vasc Biol.* 1998;18:842–851.
10. Ramos CL, Huo Y, Jung U, Ghosh S, Manka DR, Sarembock IJ, Ley K. Direct demonstration of P-selectin- and VCAM-1-dependent mononuclear cell rolling in early atherosclerotic lesions of apolipoprotein E-deficient mice. *Circ Res.* 1999;84:1237–1244.
11. Davies MJ, Gordon JL, Gearing AJ, Pigott R, Woolf N, Katz D, Kyriakopoulos A. The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. *J Pathol.* 1993;171:223–229.
12. Cybulsky MI, Gimbrone MA Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science.* 1991;251:788–791.
13. Li AC, Glass CK. The macrophage foam cell as a target for therapeutic intervention. *Nat Med.* 2002;8:1235–1242.
14. Feng B, Yao PM, Li Y, Devlin CM, Zhang D, Harding HP, Sweeney M, Rong JX, Kuriakose G, Fisher EA, Marks AR, Ron D, Tabas I. The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. *Nat Cell Biol.* 2003;5:781–792.
15. Burke AP, Farb A, Malcom GT, Liang YH, Smialek J, Virmani R. Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. *N Engl J Med.* 1997;336:1276–1282.
16. Davies MJ, Richardson PD, Woolf N, Katz DR, Mann J. Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth muscle cell content. *Br Heart J.* 1993;69:377–381.
17. Shah PK, Falk E, Badimon JJ, Fernandez-Ortiz A, Mailhac A, Villareal-Levy G, Fallon JT, Regnstrom J, Fuster V. Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques. Potential role of matrix-degrading metalloproteinases and implications for plaque rupture. *Circulation.* 1995;92:1565–1569.

18. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest*. 1994;94:2493–2503.
19. Wilcox JN, Smith KM, Schwartz SM, Gordon D. Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. *Proc Natl Acad Sci U S A*. 1989;86:2839–2843.
20. 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.
21. Lindner JR, Song J, Christiansen J, Klibanov AL, Xu F, Ley K. Ultrasound assessment of inflammation and renal tissue injury with microbubbles targeted to P-selectin. *Circulation*. 2001;104:2107–2112.
22. 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.
23. Sipkins DA, Gijbels K, Tropper FD, Bednarski M, Li KC, Steinman L. ICAM-1 expression in autoimmune encephalitis visualized using magnetic resonance imaging. *J Neuroimmunol*. 2000;104:1–9.
24. Weller GE, Lu E, Csikari MM, Klibanov AL, Fischer D, Wagner WR, Villanueva FS. Ultrasound imaging of acute cardiac transplant rejection with microbubbles targeted to intercellular adhesion molecule-1. *Circulation*. 2003;108:218–224.
25. Sipkins DA, Cheresh DA, Kazemi MR, Nevin LM, Bednarski MD, Li KC. Detection of tumor angiogenesis in vivo by alphaVbeta3-targeted magnetic resonance imaging. *Nat Med*. 1998;4:623–626.
26. Tsimikas S, Palinski W, Halpern SE, Yeung DW, Curtiss LK, Witztum JL. Radiolabeled MDA2, an oxidation-specific, monoclonal antibody, identifies native atherosclerotic lesions in vivo. *J Nucl Cardiol*. 1999;6:41–53.
27. Briley-Saebo KC, Shaw PX, Mulder WJM, Choi S-H, Vucic E, Aguinaldo JGS, Witztum JL, Fuster V, Tsimikas S, Fayad ZA. Targeted molecular probes for imaging atherosclerotic lesions with magnetic resonance using antibodies that recognize oxidation-specific epitopes. *Circulation*. 2008;117:3206–3215.
28. Lipinski MJ, Amirbekian V, Frias JC, Aguinaldo JG, Mani V, Briley-Saebo KC, Fuster V, Fallon JT, Fisher EA, Fayad ZA. MRI to detect atherosclerosis with gadolinium-containing immunonanoparticles targeting the macrophage scavenger receptor. *Magn Reson Med*. 2006;56:601–610.
29. Hwang WY, Foote J. Immunogenicity of engineered antibodies. *Methods*. 2005;36:3–10.
30. Schwarz M, Meade G, Stoll P, Ylance J, Bassler N, Chen YC, Hagemeyer CE, Ahrens I, Moran N, Kenny D, Fitzgerald D, Bode C, Peter K. Conformation-specific blockade of the integrin GPIIb/IIIa: a novel anti-platelet strategy that selectively targets activated platelets. *Circ Res*. 2006;99:25–33.
31. von Zur Muhlen C, Peter K, Ali ZA, Schneider JE, McAteer MA, Neubauer S, Channon KM, Bode C, Choudhury RP. Visualization of activated platelets by targeted magnetic resonance imaging utilizing conformation-specific antibodies against glycoprotein IIb/IIIa. *J Vasc Res*. 2008;46:6–14.
32. 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}{beta}3-integrin-targeted nanoparticles. *Circulation*. 2003;108:2270–2274.
33. Klibanov AL, Rychak JJ, Yang WC, Alikhani S, Li B, Acton S, Lindner JR, Ley K, Kaul S. Targeted ultrasound contrast agent for molecular imaging of inflammation in high-shear flow. *Contrast Media Mol Imaging*. 2006;1:259–266.
34. Rodgers SD, Camphausen RT, Hammer DA. Sialyl Lewis(x)-mediated, PSGL-1-independent rolling adhesion on P-selectin. *Biophys J*. 2000;79:694–706.
35. Sibson NR, Blamire AM, Bernades-Silva M, Laurent S, Boutry S, Muller RN, Styles P, Anthony DC. MRI detection of early endothelial activation in brain inflammation. *Magn Reson Med*. 2004;51:248–252.
36. van Kasteren SI, Kramer HB, Jensen HH, Campbell SJ, Kirkpatrick J, Oldham NJ, Anthony DC, Davis BG. Expanding the diversity of chemical protein modification allows post-translational mimicry. *Nature*. 2007;446:1105–1109.
37. Brown MH, Boles K, van der Merwe PA, Kumar V, Mathew PA, Barclay AN. 2B4, the natural killer and T cell immunoglobulin superfamily surface protein, is a ligand for CD48. *J Exp Med*. 1998;188:2083–2090.
38. Weller GE, Villanueva FS, Tom EM, Wagner WR. Targeted ultrasound contrast agents: in vitro assessment of endothelial dysfunction and multi-targeting to ICAM-1 and sialyl Lewisx. *Biotechnol Bioeng*. 2005;92:780–788.
39. Kelly K, Nahrendorf M, Yu A, Reynolds F, Weissleder R. In vivo phage display selection yields atherosclerotic plaque targeted peptides for imaging. *Mol Imaging Biol*. 2006;8:201.
40. 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.
41. 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.
42. von zur Muhlen C, von Elverfeldt D, Moeller JA, Choudhury RP, Paul D, Hagemeyer CE, Olschewski M, Becker A, Neudorfer I, Bassler N, Schwarz M, Bode C, Peter K. Magnetic resonance imaging contrast agent targeted toward activated platelets allows in vivo detection of thrombosis and monitoring of thrombolysis. *Circulation*. 2008;118:258–267.
43. von Zur Muhlen C, Sibson NR, Peter K, Campbell SJ, Wilainam P, Grau GE, Bode C, Choudhury RP, Anthony DC. A contrast agent recognizing activated platelets reveals murine cerebral malaria pathology undetectable by conventional MRI. *J Clin Invest*. 2008;118:1198–1207.
44. Chen J, Tung C-H, Mahmood U, Ntziachristos V, Gyurko R, Fishman MC, Huang PL, Weissleder R. In vivo imaging of proteolytic activity in atherosclerosis. *Circulation*. 2002;105:2766–2771.
45. Jaffer FA, Kim D-E, Quinti L, Tung C-H, Aikawa E, Pande AN, Kohler RH, Shi G-P, Libby P, Weissleder R. Optical visualization of cathepsin K activity in atherosclerosis with a novel, protease-activatable fluorescence sensor. *Circulation*. 2007;115:2292–2298.
46. Deguchi JO, Aikawa M, Tung CH, Aikawa E, Kim DE, Ntziachristos V, Weissleder R, Libby P. Inflammation in atherosclerosis: visualizing matrix metalloproteinase action in macrophages in vivo. *Circulation*. 2006;114:55–62.
47. Weissleder R, Ntziachristos V. Shedding light onto live molecular targets. *Nat Med*. 2003;9:123–128.
48. Nair A, Kuban BD, Tuzcu EM, Schoenhagen P, Nissen SE, Vince DG. Coronary plaque classification with intravascular ultrasound radiofrequency data analysis. *Circulation*. 2002;106:2200–2206.
49. Yabushita H, Bouma BE, Houser SL, Aretz HT, Jang I-K, Schlendorf KH, Kauffman CR, Shishkov M, Kang D-H, Halpern EF, Tearney GJ. Characterization of human atherosclerosis by optical coherence tomography. *Circulation*. 2002;106:1640–1645.
50. Correia LC, Atalar E, Kelemen MD, Ocali O, Hutchins GM, Fleg JL, Gerstenblith G, Zerhouni EA, Lima JA. Intravascular magnetic resonance imaging of aortic atherosclerotic plaque composition. *Arterioscler Thromb Vasc Biol*. 1997;17:3626–3632.
51. Larose E, Yeghiazarians Y, Libby P, Yucel EK, Aikawa M, Kacher DF, Aikawa E, Kinlay S, Schoen FJ, Selwyn AP, Ganz P. Characterization of human atherosclerotic plaques by intravascular magnetic resonance imaging. *Circulation*. 2005;112:2324–2331.
52. Jaffer FA, Vinegoni C, John MC, Aikawa E, Gold HK, Finn AV, Ntziachristos V, Libby P, Weissleder R. Real-time catheter molecular sensing of inflammation in proteolytically active atherosclerosis. *Circulation*. 2008;118:1802–1809.
53. Sarai M, Hartung D, Petrov A, Zhou J, Narula N, Hofstra L, Kolodgie F, Isobe S, Fujimoto S, Vanderheyden J-L, Virmani R, Reutelingsperger C, Wong ND, Gupta S, Narula J. Broad and specific caspase inhibitor-induced acute repression of apoptosis in atherosclerotic lesions evaluated by radiolabeled annexin A5 imaging. *J Am Coll Cardiol*. 2007;50:2305.
54. Kircher MF, Grimm J, Swirski FK, Libby P, Gerszten RE, Allport JR, Weissleder R. Noninvasive in vivo imaging of monocyte trafficking to atherosclerotic lesions. *Circulation*. 2008;117:388–395.
55. Yun M, Yeh D, Araujo LI, Jang S, Newberg A, Alavi A. F-18 FDG uptake in the large arteries: a new observation. *Clin Nucl Med*. 2001;26:314–319.
56. Rudd JHF, Warburton EA, Fryer TD, Jones HA, Clark JC, Antoun N, Johnstrom 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.

57. Rudd JHF, Myers KS, Bansilal S, Machac J, Rafique A, Farkouh M, Fuster V, Fayad ZA. ¹⁸F-fluorodeoxyglucose positron emission tomography imaging of atherosclerotic plaque inflammation is highly reproducible: implications for atherosclerosis therapy trials. *J Am Coll Cardiol*. 2007;50:892.
58. Tawakol A, Migrino RQ, Bashian GG, Bedri S, Vermynen D, Cury RC, Yates D, LaMuraglia GM, Furie K, Houser S, Gewirtz H, Muller JE, Brady TJ, Fischman AJ. In vivo ¹⁸F-fluorodeoxyglucose positron emission tomography imaging provides a noninvasive measure of carotid plaque inflammation in patients. *J Am Coll Cardiol*. 2006;48:1818.
59. Tahara N, Kai H, Ishibashi M, Nakaura H, Kaida H, Baba K, Hayabuchi N, Imaizumi T. Simvastatin attenuates plaque inflammation: evaluation by fluorodeoxyglucose positron emission tomography. *J Am Coll Cardiol*. 2006;48:1825–1831.
60. Aime S, Cabella C, Colombatto S, Geninatti Crich S, Gianolio E, Maggioni F. Insights into the use of paramagnetic Gd(III) complexes in MR-molecular imaging investigations. *J Magn Reson Imaging*. 2002;16:394–406.
61. Briley-Saebo KC, Mulder WJ, Mani V, Hyafil F, Amirbekian V, Aguinaldo JG, Fisher EA, Fayad ZA. Magnetic resonance imaging of vulnerable atherosclerotic plaques: current imaging strategies and molecular imaging probes. *J Magn Reson Imaging*. 2007;26:460–479.
62. Sosnovik DE, Nahrendorf M, Weissleder R. Magnetic nanoparticles for MR imaging: agents, techniques and cardiovascular applications. *Basic Res Cardiol*. 2008;103:122–130.
63. Shapiro EM, Skrtic S, Sharer K, Hill JM, Dunbar CE, Koretsky AP. MRI detection of single particles for cellular imaging. *PNAS*. 2004;101:10901–10906.
64. Shapiro EM, Skrtic S, Koretsky AP. Sizing it up: cellular MRI using micron-sized iron oxide particles. *Magn Reson Med*. 2005;53:329–338.
65. McAteer M, Sibson N, Von zur Muhlen C, Schneider J, Lowe A, Warrick N, Channon K, Anthony D, Choudhury R. In vivo magnetic resonance imaging of acute brain inflammation using micro-particles of iron oxide. *Nat Med*. 2007;13:1253–1258.
66. Weissleder R, Moore A, Mahmood U, Bhorade R, Benveniste H, Chiocca EA, Basilion JP. In vivo magnetic resonance imaging of transgene expression. *Nat Med*. 2000;6:351–355.
67. Chen JW, Pham W, Weissleder R, Bogdanov A Jr. Human myeloperoxidase: a potential target for molecular MR imaging in atherosclerosis. *Magn Reson Med*. 2004;52:1021–1028.
68. 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.
69. Lindner JR, Song J, Xu F, Klibanov AL, Singbartl K, Ley K, Kaul S. Noninvasive ultrasound imaging of inflammation using microbubbles targeted to activated leukocytes. *Circulation*. 2000;102:2745–2750.
70. Villanueva FS, Jankowski RJ, Klibanov S, Pina ML, Alber SM, Watkins SC, Brandenburger GH, Wagner WR. Microbubbles targeted to intercellular adhesion molecule-1 bind to activated coronary artery endothelial cells. *Circulation*. 1998;98:1–5.
71. Tsutsui JM, Xie F, Cano M, Chomas J, Phillips P, Radio SJ, Lof J, Porter TR. Detection of retained microbubbles in carotid arteries with real-time low mechanical index imaging in the setting of endothelial dysfunction. *J Am Coll Cardiol*. 2004;44:1036–1046.
72. 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.
73. 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*1. *J Am Coll Cardiol*. 2004;43:453–460.
74. Kooi ME, Cappendijk VC, Cleutjens KBJM, Kessels AGH, Kitslaar PJEHM, Borgers M, Frederik PM, Daemen MJAP, van Engelsehoven JMA. Accumulation of ultrasmall superparamagnetic particles of iron oxide in human atherosclerotic plaques can be detected by in vivo magnetic resonance imaging. *Circulation*. 2003;107:2453–2458.
75. Trivedi RA, U-King-Im J-M, Graves MJ, Cross JJ, Horsley J, Goddard MJ, Skepper JN, Quartey G, Warburton E, Joubert I, Wang L, Kirkpatrick PJ, Brown J, Gillard JH. In vivo detection of macrophages in human carotid atheroma: temporal dependence of ultrasmall superparamagnetic particles of iron oxide-enhanced MRI. *Stroke*. 2004;35:1631–1635.
76. Kang HW, Josephson L, Petrovsky A, Weissleder R, Bogdanov A, Jr. Magnetic resonance imaging of inducible E-selectin expression in human endothelial cell culture. *Bioconj Chem*. 2002;13:122–127.
77. Wu YL, Ye Q, Foley LM, Hitchens TK, Sato K, Williams JB, Ho C. In situ labeling of immune cells with iron oxide particles: an approach to detect organ rejection by cellular MRI. *Proc Natl Acad Sci U S A*. 2006;103:1852–1857.
78. Briley-Saebo K, Björnerud A, Grant D, Ahlstrom Hk, Berg T, Kindberg Gr. Hepatic cellular distribution and degradation of iron oxide nanoparticles following single intravenous injection in rats: implications for magnetic resonance imaging. *Cell Tissue Res*. 2004;316:315.
79. 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.
80. Cormode DP, Briley-Saebo KC, Mulder WJ, Aguinaldo JG, Barazza A, Ma Y, Fisher EA, Fayad ZA. An ApoA-I mimetic peptide high-density-lipoprotein-based MRI contrast agent for atherosclerotic plaque composition detection. *Small*. 2008;4:1437–1444.
81. Briley-Saebo KC, Johansson LO, Hustvedt SO, Haldorsen AG, Björnerud A, Fayad ZA, Ahlstrom HK. Clearance of iron oxide particles in rat liver: effect of hydrated particle size and coating material on liver metabolism. *Invest Radiol*. 2006;41:560–571.
82. Weissleder R, Stark DD, Engelstad BL, Bacon BR, Compton CC, White DL, Jacobs P, Lewis J. Superparamagnetic iron oxide: pharmacokinetics and toxicity. *AJR Am J Roentgenol*. 1989;152:167–173.
83. Wickline SA, Lanza GM. Nanotechnology for molecular imaging and targeted therapy. *Circulation*. 2003;107:1092–1095.
84. Wickline SA, Neubauer AM, Winter P, Caruthers S, Lanza G. Applications of nanotechnology to atherosclerosis, thrombosis, and vascular biology. *Arterioscler Thromb Vasc Biol*. 2006;26:435–441.
85. Kolodgie FD, John M, Khurana C, Farb A, Wilson PS, Acampado E, Desai N, Soon-Shiong P, Virmani R. Sustained reduction of in-stent neointimal growth with the use of a novel systemic nanoparticle paclitaxel. *Circulation*. 2002;106:1195–1198.
86. Joner M, Morimoto K, Kasukawa H, Steigerwald K, Merl S, Nakazawa G, John MC, Finn AV, Acampado E, Kolodgie FD, Gold HK, Virmani R. Site-specific targeting of nanoparticle prednisolone reduces in-stent restenosis in a rabbit model of established atheroma. *Arterioscler Thromb Vasc Biol*. 2008;28:1960–1966.
87. Lanza GM, Yu X, Winter PM, Abendschein DR, Karukstis KK, Scott MJ, Chinen LK, Fuhrhop RW, Scherrer DE, Wickline SA. Targeted antiproliferative drug delivery to vascular smooth muscle cells with a magnetic resonance imaging nanoparticle contrast agent: implications for rational therapy of restenosis. *Circulation*. 2002;106:2842–2847.
88. 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.
89. Sy JC, Seshadri G, Yang SC, Brown M, Oh T, Dikalov S, Murthy N, Davis ME. Sustained release of a p38 inhibitor from non-inflammatory microspheres inhibits cardiac dysfunction. *Nat Mater*. 2008;7:863–868.
90. Flacke S, Fischer S, Scott MJ, Fuhrhop RJ, Allen JS, McLean M, Winter P, Sicard GA, Gaffney PJ, Wickline SA, Lanza GM. Novel MRI contrast agent for molecular imaging of fibrin: implications for detecting vulnerable plaques. *Circulation*. 2001;104:1280–1285.