Applications of Nanotechnology to Atherosclerosis, Thrombosis, and Vascular Biology

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Abstract—The role of nanotechnology in cardiovascular diagnosis is expanding rapidly. The goal of this brief review is to illustrate selected examples of nanosystems that have been applied to the arenas of atherosclerosis, thrombosis, and vascular biology. The technologies for producing targeted nanosystems are multifarious and reflect end uses in many cases. The results to date indicate rapid growth of interest and capability in the field. The future of cardiovascular diagnosis already is being impacted by nanosystems that can both diagnose pathology and treat it with targeted delivery systems. (Arterioscler Thromb Vasc Biol. 2006;26:435-441.)

Key Words: nanotechnology ■ contrast agents ■ imaging ■ drug therapy

Advances in cellular and molecular biology are extending the horizons of medical imaging from gross anatomic description toward delineation of cellular and biochemical signaling processes. The emerging fields of cellular and molecular imaging are converging to allow noninvasive detection of the molecular components of pathological processes, such as image-based identification of specific molecules associated with inflammation or angiogenesis. Techniques have been developed recently to achieve molecular and cellular imaging with most imaging modalities, including nuclear,1 optical,2 ultrasound,3,4 and magnetic resonance imaging (MRI).5,6 In general, these methods might be considered the nondestructive in vivo analog of traditional immunocytochemistry.7 This brief review focuses on both advanced imaging methods and on new targeted nanoparticle contrast agents for early characterization of atherosclerosis and cardiovascular pathology at the cellular and molecular levels that might represent the next frontier for combining imaging and rational drug delivery to facilitate personalized medicine.8

The rapid growth of nanotechnology and nanoscience could greatly expand the clinical opportunities for molecular imaging.6,8 Nanotechnology seeks to develop and combine new materials by precisely engineering atoms and molecules to yield new molecular assemblies on the scale of individual cells, organelles, or even smaller components, generally in the range of 5 to 500 nm. The specific organization of such nanoscale materials is anticipated to confer unique chemical and biological properties on the basis of interactions that occur at their surfaces. Synthesis of such materials may occur from a “top down” approach by miniaturizing existing microscopic materials, or from a “bottom up” approach involving “self assembly” of molecules into reproducible and well-defined nanoscale constructs.

Nanoparticle Classes

Liposomes, 50- to 700-nm uni- or multilamellar vesicles comprising lipid bilayer membranes surrounding an aqueous interior, have been approved for enhancing the efficacy and safety of drugs such as doxorubicin (eg, Doxil, ALZA Corporation, Tibotec Therapeutics). Applications of liposomal technology as molecular imaging agents have been reported for both ultrasound and MRI.9,10

Emulsions, which are chemically distinct from liposomes, are oil-in-water type mixtures that are stabilized with surfactants to maintain size and shape. Perfluorocarbon core emulsions (200 to 400 nm) have been used for molecular imaging with MRI, ultrasound, fluorescence, nuclear and computed tomography imaging.4–6,11 For example, by incorporating vast numbers of paramagnetic gadolinium complexes (≥50 000) onto emulsion particles, the signal enhancement possible for each binding site is magnified dramatically by a factor of >106 over conventional paramagnetic extracellular contrast agents.12,30 Modified micellar particles such as high-density lipoprotein (HDL) or low-density lipoprotein particles have been used as molecular imaging agents for MRI.14,15

Polymers (40 to 200 nm) offer a wide variety of flexible “designer approaches” to construction of molecular imaging agents and therapeutic delivery devices.16 Size and shape can be tightly controlled, and functionalization of their surface permits binding of myriad targeting and therapeutic moieties for imaging, as well as drug and gene delivery. Polymers made from poly hydroxy acids such as the copolymer of poly

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Carbon nanotubes and fullerenes (4 nm) have been used as other compounds to inhibit aggregation and enhance stability for use as passive or active targeting agents. The iron in monocrystalline iron oxide nanoparticles, small particles of iron oxide (50 to 500 nm), or ultrasmall particles of iron oxide (USPIOs, 10 to 50 nm) produces strong local disruptions in the magnetic field of MRI scanners, which leads increased T$_2^*$ relaxation, causing a decrease in image intensity in areas with iron particle accumulation (termed “susceptibility” effects). These particles exhibit a very long circulating half-life (≥24 hours) and have been used for passive targeted imaging of pathological inflammatory processes such as unstable atherosclerotic plaques by MRI. Other metal-based agents such as gold shell nanoparticles (≈120 nm) have been used for both imaging and therapy. Carbon nanotubes and fullerenes (4 nm) have been used as particulate systems whose surfaces also can be functionalized for tissue binding. Native fluorescent properties have been reported. Quantum dots (2 to 8 nm) are constructed from semiconductor materials (eg, cadmium selenide) that manifest stable (nonquenching) fluorescent properties at various wavelengths depending on exact composition. For use in vivo, they must be coated with materials (polymers) that allow solubilization while also preventing leaching of the toxic heavy metals.

**Specific Examples of Cardiovascular Imaging**

**Atherosclerotic Plaque**

A sine qua non of the disrupted plaque is fibrin deposition. Not only is fibrin deposition one of the earliest signs of plaque rupture or erosion, but along with intraplaque hemorrhage, it also forms a considerable part of the core of growing lesions. The diagnosis of disrupted plaque by detection of small deposits of fibrin in erosions or microfractures could allow characterization of a potential “culprit” lesion before a high-grade stenosis has been formed that is selectable by cardiac catheterization.

The possibility of nanoparticle-targeted fibrin imaging with either ultrasound or paramagnetic MR contrast agents was first demonstrated by Lanza et al as early as 1996. In this case, the ligand comprised an antibody fragment highly specific for certain cross-linked fibrin peptide domains, which can be complexed to the particle either through avidin-biotin linkages or covalently to the functionalized nanoparticle as has been shown for tissue factor targeting.

For ultrasound imaging, thrombi formed in situ in canine carotid arteries were detectable within 30 minutes with commercially available 7.5-MHz linear array imaging transducers. Tissue factor is a prothrombotic transmembrane glycoprotein expressed within plaques that is upregulated after vascular injury or stent placement and that contributes as a mitogen for endothelial cells. Tissue factor imaging has been demonstrated in vivo for molecular imaging with ultrasound (Figure 1) and in vitro with MRI. The ability to image tissue factor-targeted paramagnetic nanoparticles bound to smooth muscle cell monolayers in cell culture at 1.5T attests to the potency of nanoparticles agents that carry 50 000 or more gadolinium chelates.

Echogenic liposomes, in contrast to nanoparticles or emulsions, are composed of alternating layers of aqueous fluid and lipid bilayers that are formulated to produce an ultrasound signal. Hamilton et al used these liposomes to target thrombi and various vascular signatures associated with atheroma development in injured vessels of miniswine for intravascular ultrasound imaging (Figure 1). By targeting intercellular adhesion molecule-1, vascular cell adhesion molecule-1, fibrin, fibrinogen, and tissue factor, they were able to produce targeted enhancement in the vessel walls 5 minutes after intravenous administration of the liposomes. MR imaging of vascular cell adhesion molecule also has been reported recently with the use of peptide-targeted superparamagnetic nanoparticles in aortas of apolipoprotein E null mice by Kelly et al.

For MRI, perfluorocarbon particles loaded with 50 to 90 000 gadolinium atoms per particle yielded a substantial amplification of signal from fibrin clots at 1.5T both in vitro and in vivo (Figure 2). Furthermore, the detection of disrupted plaque was illustrated in actual human carotid endarterectomy specimens obtained from patients symptomatic with transient ischemic attacks, stroke, or bruises (Figure 2). Epix Pharmaceuticals more recently has used phage display methods to produce a peptide ligand specific for fibrin (EP-2104R), which may be useful for imaging thrombi in various body locations such as the left atrium, pulmonary arteries, or coronary arteries in experimental preparations.

**Angiogenesis**

The α$_3$β$_3$-integrin is a general marker of angiogenesis and plays an important role in a wide variety of disease states, including atherosclerosis. The α$_3$β$_3$-integrin is a well-characterized heterodimeric adhesion molecule that is widely expressed by endothelial cells, monocytes, fibroblasts, and vascular smooth muscle cells, and it plays a critical part in smooth muscle cell migration and cellular adhesion, both of which are required for the formation of new blood vessels. The α$_3$β$_3$-integrin is expressed on the luminal surface of activated endothelial cells but not on mature quiescent cells. The utility of α$_3$β$_3$-integrin–targeted nanoparticles has been shown for the detection and characterization of angiogenesis associated with growth factor expression, tumor growth, and atherosclerosis.

Angiogenesis plays a critical role in plaque growth and rupture. In regions of atherosclerotic lesions, angiogenic
vessels proliferate from the vasa vasorum to meet the high metabolic demands of plaque growth. Molecular imaging of expanded vasa vasorum in atherosclerotic lesions in cholesterol-fed rabbits was first demonstrated for MRI by Winter et al with the use of paramagnetic nanoparticles targeted to integrin-expressing endothelial cells (Figure 3). Animals on a control diet exhibited no increased signal and background was minimal. Expression of integrins in the adventitial layer and beyond was confirmed by colocalized histological staining of integrin and platelet endothelial cell adhesion molecule, which is a general endothelial marker.

**Other Plaque Components**

Macrophage imaging with the use of nontargeted USPIOs was reported first by Schmitz et al in Watanabe rabbits and by Ruehm et al in cholesterol-fed atherosclerotic rabbits. Because macrophages are abundant in plaques throughout the vascular tree and are well known to ingest particulate matter, the use of superparamagnetic agents to delineate macrophages and foam cells has been pursued in both animal models and in clinical trials. The demonstration of macrophage targeting in vivo in rabbits required a waiting period of 1 to 3 days to allow for both passive uptake of sufficient numbers of particles and for bloodstream clearance of the long circulating particles. In general, the susceptibility artifacts produced extended beyond the confines of the plaque macrophages and appeared as heterogeneously distributed signal voids up and down the aorta.

In similar clinical trials of patients undergoing carotid endarterectomy by Kooi et al and by Trivedi et al, USPIO particles accumulated in the macrophages in plaques and

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**Figure 1.** Ultrasound molecular imaging with nanoparticles. Left, top panels, Tissue factor (TF)-targeted nanoparticles binding to tissue factor constitutively expressed on porcine endothelial cells in vitro. Bottom panels, Imaging the porcine carotid artery with 30 MHz intravascular ultrasound (IVUS) catheter after balloon injury. Note contrast enhancement heterogeneously distributed throughout media of vessel representing binding of TF-targeted nanoparticles to medial smooth muscle cells expressing tissue factor epitopes (left), and no enhancement in control injured segments treated with untargeted nanoparticles. Reprinted with permission from Lanza et al and Lippincott Williams &Wilkins.

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**Figure 2.** MRI of thrombi with paramagnetic nanoparticles targeted to fibrin. Left, Thrombus formed in canine jugular vein imaged with T1 weighted pulse sequences at 1.5T. Right, “Disrupted” carotid endarterectomy specimens incubated with fibrin-targeted nanoparticles binding to small amounts of fibrin at shoulders of ruptured plaque cap imaged in vitro at 1.5T. Reprinted with permission from Lippincott Williams & Wilkins.
were optimally imaged as signal reductions at 24 hours after injection. Kooi et al also noted that more contrast change was observed for ruptured plaques than for stable plaques: USPIO-labeled macrophages have been imaged and localized to unstable and ruptured plaques (75% demonstrating uptake) but not in stable lesions (only 7% showing USPIO uptake).52

Recently, Frias et al14 reported the development of recombinant paramagnetic HDL-like particles that have been shown to enhance atherosclerotic regions in apolipoprotein E-deficient mice. These particles are formed through the delipidation of normal isolated human HDL particles, followed by reconstitution with phospholipids and addition of a phospholipid-based conjugate of Gd-DTPA (15 to 20 molecules of gadolinium included in each 9 nm particle) for signal enhancement. Nonselective accumulation in atherosclerosis has been demonstrated.

Fayad et al54 have also demonstrated the use of other nontargeted agents such as gadofluorine, a lipophilic chelate of gadolinium that forms 5-nm micelles in aqueous solution, to preferentially label the fatty cores of plaques. The small size and lipophilic nature of this contrast agent allows it to accumulate in lipid rich areas of plaque in cholesterol-fed rabbits.

**Stem Cell Imaging**

Stem cell imaging with MRI is another emerging area that might fit under the rubric of molecular imaging with targeted nanoparticle contrast agents. Cells can be treated with superparamagnetic nanoparticles in vitro and then engrafted into the selected location by local injection. The stem cells ingest nanoparticles through endocytosis by various strategies, including coating the particles with dendrimers, transfection agents, or antibodies/peptides,55–57 which results in the intracellular accumulation of significant amounts of intact nanoparticles that then can exert a local susceptibility effect for detection in vivo. These particles appear to be well tolerated by cells over the long term, although the signal ultimately dissipates as the cells divide and distribute the material or as the particles are catabolized naturally. Along these lines, Frank et al,56 Bulte et al,58 and others have demonstrated the utility of stem cell tracking after in vitro preparation with superparamagnetic nanoparticles. More recently, Kraitchman et al59 demonstrated the ability to detect and track mesenchymal stem cells injected into necrotic regions of a pig heart at 1.5 T (Figure 4). Recent data indicating clinical feasibility for MRI stem cell tracking was reported for labeled dendritic cells injected into lymph nodes in patients with melanoma.60

Alternatively, the fluorine component of perfluorocarbon-based nanoparticles might be used to advantage for cell imaging after particle ingestion. Our group originally demonstrated the concept of targeted nanoparticle fluorine imaging at 1.5T or 4.7T for detection of experimental thrombi or small fibrin deposits in disrupted human carotid arteries using nanoparticles made with perfluorocetyl bromide, crown ether,
Delivering and Monitoring Therapy

The potential dual use of nanoparticles for both imaging and site-targeted delivery of therapeutic agents to cardiovascular disease offers great promise for individualizing therapeutics. Image-based therapeutics with site-selective agents should enable conclusive assurance that the drug is reaching the intended target and a molecular effect is occurring. As an example of this new paradigm for drug delivery, Lanza et al.30 treated smooth muscle cells in culture with tissue factor-targeted nanoparticles that were loaded with paclitaxel. The smooth muscle cells were harvested from pig aortae and constitutively expressed tissue factor epitopes in vitro. Binding of the drug-free nanoparticles to the cells yielded no alterations in growth characteristics of the cultured cells. When paclitaxel-loaded nanoparticles were applied to the cells, however, specific binding elicited a substantial reduction in smooth muscle cell proliferation. Nontargeted paclitaxel-loaded particles applied to the cells (ie, no binding of nanoparticles to cells occurred) resulted in normal cell proliferation, indicating that selective targeting may be a requirement for effective drug delivery for these emulsions. Similar behavior has been demonstrated for doxorubicin-containing particles.30 Recent reports indicate that intravenous delivery of fumagillin-loaded nanoparticles (an antiangiogenic agent) targeted to α,β3-integrin epitopes on vasa vasorum in growing plaques results in marked inhibition of plaque angiogenesis in cholesterol fed rabbits.62 Kolodgie et al63 also used taxol-containing nontargeted albumin nanoparticles for limitation of the restenotic response after angioplasty and stent placement in experimental animals.

The unique mechanism of drug delivery for highly lipophilic agents such as paclitaxel contained within emulsions depends on close apposition between the nanoparticle carrier and the targeted cell membrane and has been described as “contact facilitated drug delivery.”730 In contrast to liposomal drug delivery (generally requiring endocytosis), the mechanism of drug transport in this case involves lipid exchange or lipid mixing between the emulsion vesicle and the targeted cell membrane,64,65 which depends on the extent and frequency of contact between 2 lipidic surfaces.30,64 The rate of lipid exchange and drug delivery can be greatly increased by the application of clinically safe levels of ultrasound energy that increase the propensity for fusion or enhanced contact between the nanoparticles and the targeted cell membrane.64,65

Limitations

Although both molecular imaging and targeted therapeutics are attractive subjects for clinical evaluation, the ultimate role of these technical advances must be established in clinical trials. To date, no solid proof of efficacy has been provided with respect to altering courses of therapy or patient outcomes. The choice of imaging modalities also is broad and remains to be worked out in practice, and it will depend on cost, availability, and the specific application. As is the case for any novel pharmacological agent undergoing clinical trials, the use of nanoparticles also will require thorough evaluation for pharmacokinetics, biodistribution, and toxicity. However, in the era of molecular medicine where early diagnosis and personalized therapeutics may reduce risk and save lives, the promise of these novel technologies and approaches represents a new avenue to disease control that appears extraordinarily compelling.

Conclusion

The combination of targeted drug delivery and molecular imaging with MRI has the potential to revolutionize the field of cardiology, as well as many other fields. Drug delivery agents that are also quantifiable at the targeted site based on imaging readouts may ultimately permit serial characterization of the molecular epitope expression and confirmation of therapeutic efficacy, thereby promoting truly personalized medical regimens. Rapid developments in genomics, molecular biology, and nanotechnology have energized the multidisciplinary field of molecular imaging, and we anticipate...
that clinical applications are at hand for these powerful agents.

References


