Nanotechnology for Molecular Imaging and Targeted Therapy

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The recent emergence of “molecular imaging” as an integrated discipline in academic medical centers has set the stage for an evolutionary leap in diagnostic imaging and therapy. Molecular imaging is not a substitute for the traditional process of image formation and interpretation, but is intended to improve diagnostic accuracy and sensitivity by providing an in vivo analog of immunocytochemistry or in situ hybridization. It is less concerned with native image contrast or resolution, which are keys for depicting the effects of the disease on surrounding normal tissues, but rather, it seeks to enhance the conspicuity of microscopic pathologies by targeting the molecular components or processes that represent actual mechanisms of disease. Moreover, imaging will become crucial for in vivo characterization of the complex behaviors of disease in time and space that will tell us: where it is, how big it is, how fast it is developing, how many molecular processes are contributing simultaneously, what to treat it with, how it is responding to therapy, and how it is changing.

Elements fundamental to the growth of molecular imaging are exemplified in summary statements from the second Biomedical Imaging Symposium: Visualizing the Future of Biology and Medicine (Available at: http://www.becon1.nih.gov/symposium1999.htm), which convened at the National Institutes for Health (NIH) on June 25 and 26, 1999, and was cosponsored by the Biomedical Engineering Consortium (BECON), the Radiological Society of North America, and the American Institute of Medical and Biological Engineering. The goals of the Symposium were to: (1) “identify the most important challenges and opportunities in biomedical imaging science,” and (2) “develop strategies for integrating imaging science with biological and medical research.” The formation of the BECON was followed by a Congressional mandate to establish a new National Institute for Biomedical Imaging and Bioengineering devoted to the development of novel technologies, including molecular imaging and therapeutics, without the traditional restrictions of hypothesis-driven research. The BECON symposium identified 5 action items critical to achieving these goals:

- Implementation of multidisciplinary research programs, especially in the area of molecular imaging or image guided therapy;
- Development of new imaging technologies, probes, and contrast agents that are the tools for linking imaging to specific biological processes;
- Initiation of new training programs in molecular imaging to create a generation of scientists for whom the principles of imaging, physics, bioengineering, molecular biology, physiology, pharmacology, and pathophysiology form an intellectual continuum;
- Planning for new clinical trials combined with informatics approaches to assess biomedical imaging technologies;
- Enhancement of cooperation among the NIH, the Food and Drug Administration (FDA), the Health Care Financing Administration (HCFA), and industry (both large and small businesses) to improve the speed with which new imaging technologies, probes, and contrast agents can be transferred into clinical practice.

The coincident expansion of “nanoscale science” already will play a fundamental role not only in molecular imaging, but also in biosensors, biomarkers, and drug delivery over the next decade. The US National Nanotechnology Initiative (see the statement by M.C. Roco, available at http://www.nano.gov/roco_vision.html) recognizes that “the relative arrangement of the elementary blocks of matter into their assemblies leads to new properties and functions even for the same chemical composition.” Ironically, as devices and agents become smaller, they will require bigger and more multidisciplinary teams to realize the anticipated revolution. In contrast to the time-honored models of highly specific and temporally limited collaborative interactions among very focused laboratories, nanoscience efforts will require that investigators learn each other’s languages in some depth and form partnerships that integrate individual intellectual components into a cohesive team approach. The complexity of the new nanotechnologies and the scope of their clinical and commercial applications requires direct and immediate access to diverse “in-house” expertise, which may dramatically impact the traditional academic paradigm for doing science with respect to conceiving new models for team organization and resource allocation.

Imaging Applications

Because molecules themselves obviously are too small to be imaged directly with noninvasive techniques, specific and sensitive site-targeted contrast agents are typically employed as beacons to depict epitopes of interest. And, unlike traditional blood pool contrast agents, a site-targeted agent is
intended to enhance a selected biomarker that otherwise might be impossible to distinguish from surrounding normal tissue. Molecular imaging actually has been a clinical reality for some time with the use of targeted radionuclides. Somatostatin-receptor imaging for detection of neuroendocrine tumors is but one clinical example, as is fluoro-DOPA glucose (FDG) imaging by PET for characterization of diverse disease states. Targeted detection of cellular apoptosis with the use of technetium-labeled annexin is now in clinical trials (Theseus, Inc) on the basis of binding to membrane phosphatidyl serine epitopes that are exposed during apoptosis. Other nuclear constructs appear useful for monitoring transfection events by imaging proteins that are expressed after reporter gene transcription. For example, herpes virus thymidine kinase genes can be used as a reporter construct in association with a therapeutic gene by phosphorylating certain exogenously supplied radiolabeled probes (or substrates) that are then trapped inside of cells where they can be imaged.3

However, the explosive growth of biocompatible nanotechnologies now promises to expand the horizon for molecular imaging and therapy with a host of novel agents. The desired properties of such targeted contrast agents are: long circulating half-life (hours), selective binding to epitopes of interest, low background signal and prominent contrast-to-noise enhancement, acceptable toxicity profile, ease of production and clinical use, applicability with standard commercially available imaging modalities, and promise for adjunctive therapeutic delivery. Clinical availability of these agents is expected to redefine the practice of imaging by focusing on cellular and molecular mechanisms of disease, which will create opportunities for more precise and rational design of conjunctive drug and/or gene delivery nanosystems.

The contrast mechanism will depend on the choice of imaging modality, which itself is determined by the clinical problem and accessibility for imaging. For example, carrier moieties such as nanoparticles (liposomes or emulsions),1–6 dendrimers,7,8 viral constructs,9 buckeyballs,10 or various polymers11,12 can be loaded with large payloads of imaging agents such as paramagnetic or superparamagnetic metals, optically active compounds (eg, fluorescent molecules), or radionuclides to enable detection with standard imaging equipment. In the case of ultrasound imaging, the intrinsic physical properties of the carrier agents themselves (density and compressibility) establish the means for detection. For certain constructs, such as liquid perfluorocarbon nanoparticles,6,13,14 considerable flexibility exists to utilize any or all imaging modalities.

The contrast agent should manifest high affinity and avidity for the target. Generally, the targeting ligands are coupled directly to the carriers and comprise monoclonal antibodies or their fragments, peptides, small molecule peptidomimetics, or aptamers, which confer specificity of binding. Dissociation constants in the nanomolar range or better are preferred. Multivalent binding can be useful to enhance avidity and reduce “off-rates” so that binding persists long enough to permit imaging at convenient times after delivery of the agent. Polyvalent binding is possible with the use of more than one ligand type per carrier, or with mixtures of ligand-carrier constructs directed at different targets.

To illustrate such nanosystems, a few examples of MRI and ultrasound applications in molecular imaging are cited. Paramagnetic polymerized liposomes bearing antibody ligands to neovascular integrins αβ have been used for targeting experimental tumor angiogenesis, and accumulation after 24 hours allows sufficient signal for imaging. Similarly, liquid perfluorocarbon nanoparticle emulsions targeted to αβ also permit robust tumor imaging after only 1 hour in the circulation, with specific uptake demonstrated by in vivo competition experiments.15 Indeed, liquid perfluorocarbon nanoparticles were the first example of molecular targeting agents broadly useful for MRI of thrombi by incorporating antibody ligands directed against cross-linked fibrin,14 and they have been shown recently to enable characterization of experimental thrombi in vivo and human unstable carotid plaques ex vivo.16

For paramagnetic agents, it is important that the longitudinal relaxivity per molecular binding site for the complex is maximized, allowing contrast enhancement with very small numbers of paramagnetic particles. For paramagnetic perfluorocarbon nanoparticles, for example, particle-based relaxivities (or “unit signal strength”) are the highest reported to date in the literature, providing a sufficient signal to be detectable at local concentrations in the picomolar range.16 Even single cells may be imaged with such agents.17 However, for epitopes in much higher concentrations, such as fibrin in thrombi, paramagnetic molecular imaging agents with more modest enhancements in relaxivities could be useful for targeting.

“Susceptibility” or “cold spot” imaging agents have been produced by combinations of carriers with iron oxides (eg, ultra-small particles of iron oxide) or alternative lanthanide species.18,19 Recent work has demonstrated potential for delineation of early atherosclerosis in experimental models by uptake of ultra-small particles of iron oxide in plaque macrophages.20 Stem-cell labeling with magneto-dendrimers permits MRI detection and precise localization after therapeutic injection.21 Nanoscale polymers might be utilized as carriers of magnetic and therapeutic materials as well.11,12 Various transport ligands, such as the retroviral “tat” protein, have been coupled to these agents to promote cell uptake (eg, cross-linked iron oxide nanoparticles).22,23

Other so-called “smart” agents are designed to take up residence in all cells of the body but to be activated only by specific enzymes that are expressed in the cell under certain pathologic states, or by the protein products (enzymes) of reporter genes after therapeutic transfection.24 Cleavage of active sites on these agents exposes sequestered gadolinium atoms to free water and facilitates rapid water exchange to produce an effect on local proton relaxation, in turn enhancing image contrast.

Targeted perfluorocarbon nanoparticles were the first reported molecular imaging agent for ultrasound applications and were shown to augment reflectivity from fibrin thrombi in vivo by 2 orders of magnitude or more.4,25,26 Additionally, targeting to vascular epitopes such as tissue factor, whose expression is induced in smooth muscle cells in vivo after
angioplasty, is possible because these particles can penetrate through microfissures into the vascular media.\textsuperscript{1,3,27} Reflective liposomes have also been used to specifically target endothelial integrins.\textsuperscript{6} Other microscale systems (stabilized perfluorocarbon gas microbubbles) have been used for molecular ultrasound imaging of thrombi,\textsuperscript{28} but generally these are restricted to the vasculature in view of their size (≈5 µm) and their susceptibility to destruction with clinical ultrasound imaging intensities.

**Therapy**

The ability to incorporate drugs or genes into detectable site-targeted nanosystems represents a new paradigm in therapeutics that could usher in an era of image-based drug delivery and dosing. Payloads of therapeutic agents, such as genes or radionuclides, can be complexed to the carriers themselves. Drugs can be linked to or dissolved within carrier lipid coatings, deposited in subsurface oil layers, or trapped within the carriers themselves. Nontargeted nanocarriers, such as solid lipid particles,\textsuperscript{29} albumin,\textsuperscript{30} or polymer nanosystems,\textsuperscript{31} have been described for transporting a wide variety of agents. However, the use of targeted agents should concentrate even greater drug amounts within selected tissues by specific uptake mechanisms, implying that serum levels of drugs could be more effectively minimized to mitigate harmful side effects.

Drug delivery to specific cells from nanocarriers can occur by diffusion, particle fusion and internalization into cells, component (lipid-lipid) exchange and convective flux, biolistic, or some combination of these mechanisms. In the case of ultrasound, methods for gene delivery with synthetic vectors such as microbubbles rely on mechanical stimulation of microbubbles that operate like miniature gene guns.\textsuperscript{32} Stabilized gaseous microbubble contrast agents (≈5 µm in diameter) have demonstrated potential for use as transfection agents by incorporating DNA directly into the bubble shell or interior.\textsuperscript{33,34} Cavitational destruction of the bubbles with focused ultrasound applied externally releases genes at selected sites. Although the exact regimens remain to be worked out, the effects of ultrasound on microbubbles at clinical frequencies and powers can potentiate carrier interactions with tissues.\textsuperscript{32}

Nanoparticles also are useful for delivery of pharmaceutical agents after binding to target cellular epitopes by a mechanism called “contact facilitated drug delivery.” Binding and close apposition to the targeted cell membrane permits enhanced lipid-exchange with the lipid monolayer of the nanoparticle, which accelerates convective flux of lipophilic drugs (e.g., paclitaxel) dissolved in the outer lipid membrane of the nanoparticles into the targeted cells.\textsuperscript{17} Such nanosystems can serve as drug depots exhibiting prolonged release kinetics and long persistence at the site. These systems can also function as synthetic vectors for gene delivery when formulated with cationic lipids, much like circulating versions of conventional transfection vehicles (e.g., Lipofectin).\textsuperscript{35}

The opportunity to confirm drug delivery and dose by imaging represents a novel feature for nanosystems that provide controlled drug release. Fortunately, the imaging signals produced by the carriers themselves, along with knowledge of the amount of the specific drug contained per particle, will allow estimation of tissue drug levels. In the case of paramagnetic nanoparticles, a range of perfluorocarbon components can be detected and quantified by NMR spectral analysis to permit quantitative differentiation among a variety of simultaneously targeted molecules.\textsuperscript{17} Targeted “quantum dot” nanosystems might also enable similar tunable, multispectral characterization, although signal strength and limited imaging depth will remain problematic.\textsuperscript{36} These possibilities ultimately might allow rational drug dosing on the basis of quantification of the local concentrations of the agent, and may eventually permit more exquisite titration of especially potent drugs that would otherwise exhibit unacceptable toxicity if employed at high serum levels.

As to the future, the growth of molecular imaging will continue to be nurtured by the academic community. The Academy of Molecular Imaging (http://www.ami.org) and the Society of Molecular Imaging (http://www.societymolecularimaging.org) were formed at about the same time to support the community of scientists interested in image-based phenotypic characterization at the cellular and molecular level. The National Cancer Institute has taken a leadership position in molecular imaging with the advent of training and research programs such as the Centers for Molecular Imaging, the Unconventional Innovations Programs, and preclinical drug development programs for novel molecular imaging agents. The National Heart, Lung, and Blood Institute is also developing programs for the support of nanosciences, molecular imaging, and biomarker research through conjoint efforts of the SPARK-2 Working Group and the various professional societies that are stakeholders in the future of health care in the United States (personal communication, October, 2002). The European Union has developed multinational initiatives through the Sixth Framework Program for research (2002 to 2006) that was adopted in June 2002, setting aside funds totaling about $2 billion over 3 years for research initiatives including “life sciences, genomics, and biotechnology for health,” as well as “nanotechnologies and nanosciences.” Corporate research programs in molecular imaging have been initiated by multinational companies such as Philips Medical Systems, Siemens, and General Electric. Many pharmaceutical industry research groups have already installed dedicated imaging laboratories to facilitate selection of candidate agents and early drug development.

Accordingly, prospects appear bright for molecular imaging and the development of biocompatible nanotechnologies. Complete characterization of the human genome and the subsequent emergence of proteomics, together with progress in basic molecular and cellular research, should continue to uncover new and useful molecular targets. In many cases, the need for new biomarkers of disease and surrogate end points for drug trials will drive these efforts. The major challenge is to understand and foster the multidisciplinary intellectual environments in academic and corporate settings that are appropriate to the tasks.

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