The AAPS Journal 2007; 9 (2) Article 19 (http://www.aapsj.org).

Themed Issue: Nanotechnology and Drug Delivery
Guest Editors - Craig K. Svensson and Alexander V. Kabanov

Nanosystems for Simultaneous Imaging and Drug Delivery to T Cells

Submitted: January 16, 2007; Accepted: May 11, 2007; Published: June 8, 2007

Tarek M. Fahmy,[1] Peter M. Fong,[1] Jason Park,[1] Todd Constable,[1,2] and W. Mark Saltzman[1]

[1]Department of Biomedical Engineering, Yale University, PO Box 208284, New Haven, CT 06511
[2]Department of Diagnostic Radiology, Yale University School of Medicine, PO Box 208034, New Haven, CT 06511

Abstract

The T-cell response defines the pathogenesis of many common chronic disease states, including diabetes, rheumatoid arthritis, and transplant rejection. Therefore, a diagnostic strategy that visualizes this response can potentially lead to early therapeutic intervention, avoiding catastrophic organ failure or prolonged sickness. In addition, the means to deliver a drug dose to those cells in situ with the same specificity used to image those cells would provide for a powerful therapeutic alternative for many disease states involving T cells. In this report, we review emerging nanosystems that can be used for simultaneous tracking and drug delivery to those cells. Because of their versatility, these systems—which combine specific receptor targeting with an imaging agent and drug delivery—are suited to both basic science and applications, from developing therapeutic strategies for autoimmune and alloimmune diseases, to noninvasive tracking of pathogenic T-cell migration.

Keywords: T cells, noninvasive imaging, drug delivery, nanoparticles

Migration of T Cells

While the normal trafficking of circulating T cells is critical for a healthy immune response, these cells occasionally become pathogenic, with disastrous consequences.[1-5] In healthy individuals, these cells traffic from circulation to tissue, then to lymphatics and back to circulation (Figure 1). They do this in high numbers and at different speeds throughout the blood and tissue.[5,6] Aberrant function at the specific sites of interaction or an abnormal migration pattern may dictate the pathogenesis of a variety of disease states. Table 1 shows some disease states associated with aberrant T-cell function and the epitopes recognized by those specific T cells during migration.

Corresponding Author: Tarek M. Fahmy, Department of Biomedical Engineering, Yale University, PO Box 208284, New Haven, CT 06511. Tel: (203) 432-1043; Fax: (203) 432-0030; E-mail: Tarek.fahmy@yale.edu

While the migration of cells through the various microenvironments is orchestrated by a complex set of interactions (which are beyond the scope of this review),[7,8] it is important to note that, for the purpose of imaging and modulation by drug delivery, T cells are moving targets. Nanosystems that bind to these targets in a specific manner may offer a powerful approach to imaging the T cells' movement and delivering a therapeutic drug dose. The ideal nanosystems, therefore, must possess certain properties (eg, small size for internalization, multivalent attachment for enhanced avidity of interaction with cells, visibility by invasive and noninvasive modalities, high drug dose–carrying capacity, control over drug release, safety) to allow for translational applications in humans.

T-Cell Targeting: Background and Limitations of Current Approaches

Specificity in the T-cell response defines the pathogenesis of many common chronic, autoimmune, and alloimmune disease states and is mediated by the interaction of the T-cell antigen receptor (TCR) with peptide-presenting major histocompatibility complex (peptide/MHC) on the antigen-presenting cell.[7,9] Thus, the TCR–peptide/MHC interaction is a critical target in the immune response. The inherent low affinity of the TCR for the peptide/MHC (1-100 um)[10,11] has precluded the use of soluble peptide MHC monomers for direct detection of antigen-specific T cells. Detection of these subsets is critical for assessing the extent of the T-cell immune response and the onset of disease. Thus, artificial constructs with multivalent peptide/MHCs, which bind the TCR with enhanced avidity, have had a profound impact on the understanding and characterization of the T-cell immune response at the experimental and clinical levels.[12-14] These dimeric and tetrameric forms of the MHC have been used effectively in quantitation and enumeration of a variety of T-cell types in different basic and clinical settings.

While the ability to track T cells and antigen-specific T cells in vitro and in vivo would be helpful for understanding the genesis of disease states involving T-cell recruitment,[14,15] the added ability to modulate these cells would lead to novel approaches for repairing immune system defects and restoring immune competence. Perhaps the most common strategy for modulating the antigen-specific T-cell response in vivo involves the induction of antigen-specific T-cell

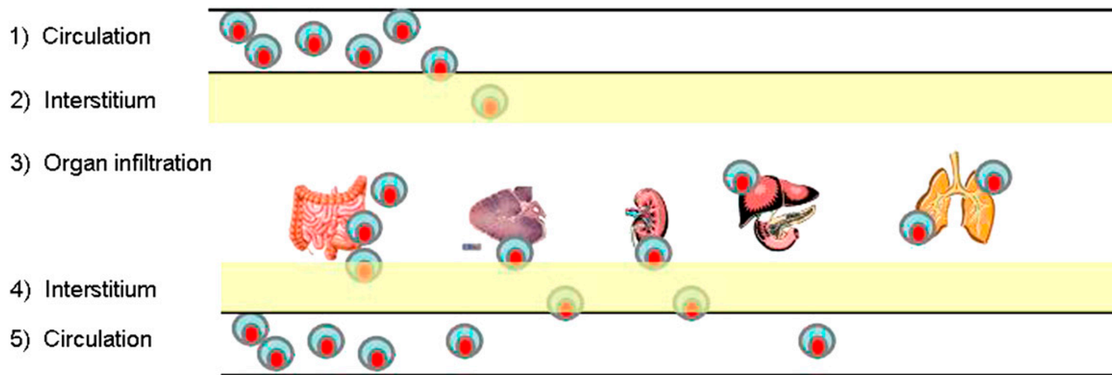

Figure 1. Schematic of lymphocyte migration through the body.

unresponsiveness or anergy by exposure to controlled doses of antibodies or peptide/major histocompatibility ligands (peptide/MHC).[16] However, in this approach, affected T cells remain alive in a hyporesponsive state that can be reversed by the absence of antigen or the presence of cytokines. Thus, in most cases a continuous anergic response requires multiple treatments with antigens. A second approach involves the conjugation of T-cell antigens to immunosuppressive drugs, which would serve to direct drug delivery to a targeted population of T cells.[17] But conjugation of drug to carrier antigens requires chemical modifications that are not always easy to achieve. In addition, the effectiveness of conjugates is often limited because it is difficult to provide unhindered antigen presentation together with effective drug delivery.

Clearly, a multifunctional molecular scaffold that delivers drug specifically to T-cell populations of interest—but in addition could be engineered for imaging of the targeted T-cell population—would offer substantial benefits over current approaches. In this review, we focus on 3 types of systems that may accomplish this goal: liposomal systems, dendrimers, and solid biodegradable nanoparticles.

Tracking T-Cell Trafficking and Migration

A variety of cellular imaging techniques have been developed to track the distribution and locomotion of T cells in vivo (Table 2). All techniques aim to increase the signal difference between the cells and the background using either an ex vivo approach (where cells are isolated and labeled with specific markers, then reintroduced into the body and tracked by a variety of modalities) or an in vivo approach (in which T cells are targeted with injection of tagged antibodies or specific ligands).

The classical technique involving histological analysis of tissue and fluorescent labeling of tissue sections with congenic markers specific for T cells has a long history of use in identifying T-cell migration to various tissue sites.[18,19] Indeed, this technique was used after sectioning a whole animal to identify global distribution of antigen-specific T cells during an immune response[20,21] and has yielded valuable information regarding the frequency and phenotypes of T-cell trafficking in response to antigen challenges. But while histology provides superior spatial resolution in local microenvironments, it is severely limited in its ability to resolve dynamic changes. In addition, the labor associated with sectioning a whole animal is enormous and costly, so this technique is not used for routine analysis in laboratory investigative work.

Less invasive, high-resolution techniques such as intravital 2-photon fluorescence microscopy have been used to track ex vivo fluorescently labeled T cells implanted into tissue.[22-24] These studies have revealed the autonomous nature of T-cell trafficking in lymph nodes and spleen: cells move by random walk rather than by guidance.[24] More important,

Table 1. Representative Disease States Involving T-Cell Recruitment to Specific Organs

Organ	Antigen	Disease State
Pancreas	Pancreatic beta-cell	Type I diabetes mellitus
Intestine	Vascular endothelium (MAdCAM)	Crohn's disease
Kidney	Glomerular immune complex deposits	Lupus nephritis
Brain	Myelin basic protein	Multiple sclerosis
Joints	Synovial joint antigen	Rheumatoid arthritis
Thyroid	TSH-R, IGF-IR, thyrocytes*	Graves' disease

TSH-R indicates thyroid stimulating hormone receptor; IGF-IR, insulin like growth factor receptor.

Table 2. Imaging Techniques for Lymphocyte Trafficking*

Technique	Imaging Modality	Advantages	Disadvantages
Histological analysis	Fluorescence Colorimetric stains	High spatial resolution Defined environment Lack of movement artifacts	Ex vivo imaging No physiological flows Extensive sample preparation
Intravital microscopy	Bright-field illumination Fluorescence	In vivo imaging Temporal and spatial resolution	Invasiveness Restrictive access for imaging Technical difficulty Hindrance by physiological artifacts such as movement
Bioluminescence PET SPECT MRI	Luminescence Tomography Magnetic resonance	Noninvasiveness Whole-body imaging Temporal and spatial resolution	Lower resolution than for intravital microscopy Lack of widespread accessibility Technical difficulty in implementation Need for contrast agent enhancement for monitoring cellular trafficking

*PET indicates positron emission tomography; SPECT, single photon emission computed tomography; MRI, magnetic resonance imaging.

the studies have shown that T cells are not stationary, waiting for chance encounters with antigen, but rather are highly motile, rapidly pacing and scanning for antigen in lymphoid organs.[24] The implications of these studies from the standpoint of cellular immunology has been extensively reviewed, and we refer the reader to those reports for further information.[7,18,22] For the purpose of this review, however, the practical application of this imaging modality in the clinic and for routine investigations in laboratory animals is limited because of its invasive nature as well as its ability to image only exposed superficial or explanted tissue.

To overcome this limitation, other imaging modalities have been investigated. For example, T cells labeled with radioisotopes such as [18F] 2-fluorodeoxyglucose[25] and 99mTc-hexamethylpropyleneamine oxime[26] have been used to study lymphocyte trafficking by positron emission tomography (PET). While this approach offers the potential to track T cells in deep tissue, it is limited in the dynamic time frame needed to visualize T-cell trafficking because of the short half-life of the isotopes. Repeated injections and the use of tracers with longer half-lives may be possible in animal models but would not be feasible for translational use in humans.

An alternative safer method for in vivo lymphocyte visualization uses bioluminescence.[27,28] Because most cells lack natural fluorescence, bioluminescence has been used to track T cells after transfection with a bioluminescent marker such as luciferase. Recent studies have documented T-cell tracking to subcutaneously implanted tumors and the trafficking of T cells to inflamed tissue in models of autoimmune disesase.[29,30] The advantage of this approach over invasive techniques is that it offers the potential for imaging T-cell trafficking deep into the body and with minimal perturbation of tissue. However, it is limited in its spatial resolution because of the poor visibility of emitted light from deep tissue and the lack of conventional instrumentation that may be used for light amplification.

Of the different imaging systems available to researchers and clinicians, magnetic resonance (MR) imaging and PET offer the best potential for noninvasive imaging deep into tissue, high sensitivity, and spatiotemporal resolution through the use of appropriate contrast agents. For a detailed comparison, please see reviews by Herschman[31] and Sosnovik.[32] In this review, we will focus specifically on the use of MR imaging for T-cell tracking. The MR signal strength is directly related to the relaxation rates of protons in the local environment (R_1, the longitudinal relaxation rate, and R_2, the transverse relaxation rate). Because of this correlation, agents that enhance the rate of either type of relaxation can be used as MR contrast agents. The 2 most widely used contrast agents for cellular tracking are gadolinium-based agents, which are T_1 contrast agents that cause positive contrast enhancement and provide brighter images on accumulation in the target site, and superparamagnetic iron oxide particles, which are T_2 contrast agents that give negative contrast enhancement and thus darker images in areas of accumulation. Because nonspecific internalization by T cells is inefficient, contrast labeling of those cells requires tagging with internalization enhancers such as peptides or specific ligands that facilitate the internalization of the contrast agent without compromising cellular function. A variety of these internalization enhancers have been proposed and used effectively, including HIV tat peptides (which can

increase T-cell uptake of liposomal[33] and iron oxide nanoparticles[34] 100-fold compared with unlabeled contrast agent), anionic iron oxide nanoparticles,[35] and transfection reagents combined with the contrast agent.[36]

But while supermagnetic iron oxide (SPIO) and gadolinium-based agents are well-established, safe, and effective MR contrast agents, little has been achieved from the standpoint of incorporating these contrast agents into drug delivery vehicles for targeting specific T cells. A variety of other nanosystems have been developed that can potentially achieve this drug delivery, including (1) liposomal-based systems; (2) macromolecular systems such as dendrimers; and (3) solid nanoparticles, such as those fabricated from biocompatible polymers (Figure 2).

NANOSYSTEMS

Liposomal Systems

Liposomes as drug delivery vehicles were first proposed by Gregoriadis[37-40] but have also been studied extensively for the purposes of noninvasive imaging (see reviews by Laverman et al,[41] Mulder et al,[42] and Torchilin[43]). Liposomes are generally composed of amphiphilic phospholipids and cholesterol that self-associate into bilayers encapsulating an aqueous interior. These can be formulated into small structures (between 80 and 100 nm) that encapsulate either hydrophilic drugs in the aqueous interior or hydrophobic drugs within the bilayer (Figure 2). Encapsulation is achieved using a variety of loading methods, most notably the pH gradient method (used for loading vincristine)[44] or the ammonium sulfate method (used for loading doxorubicin).[45] Additionally, the liposome surface can be engineered to improve its properties.[46,47] Thus far, the most noteworthy surface modification is the incorporation of the hydrophilic polymer polyethylene glycol (PEG), which serves as a barrier preventing interactions with plasma proteins, thus retarding recognition by the reticuloendothelial system[48] and enhancing the liposome circulation lifetime. As a carrier for imaging agents, liposomes have proven to be versatile as well.[43,49] For example, liposomes have been loaded with fluorochromes and targeted to both T-cell and B-cell lymphocytes for in vitro characterization. They have also been used in scintigraphic imaging of lymph nodes and inflammation[41,50] by loading with radionuclides such as technetium-99m[51] or inidium-111[52,53] as well as targeted imaging of tumor development and drug delivery to those tumors.[43,53]

The combination of targeting specificity, improved pharmacokinetic distribution, and the ability to incorporate a variety of agents into either the bilayer or the interior has led to an increased study of liposomal systems for enhancement of

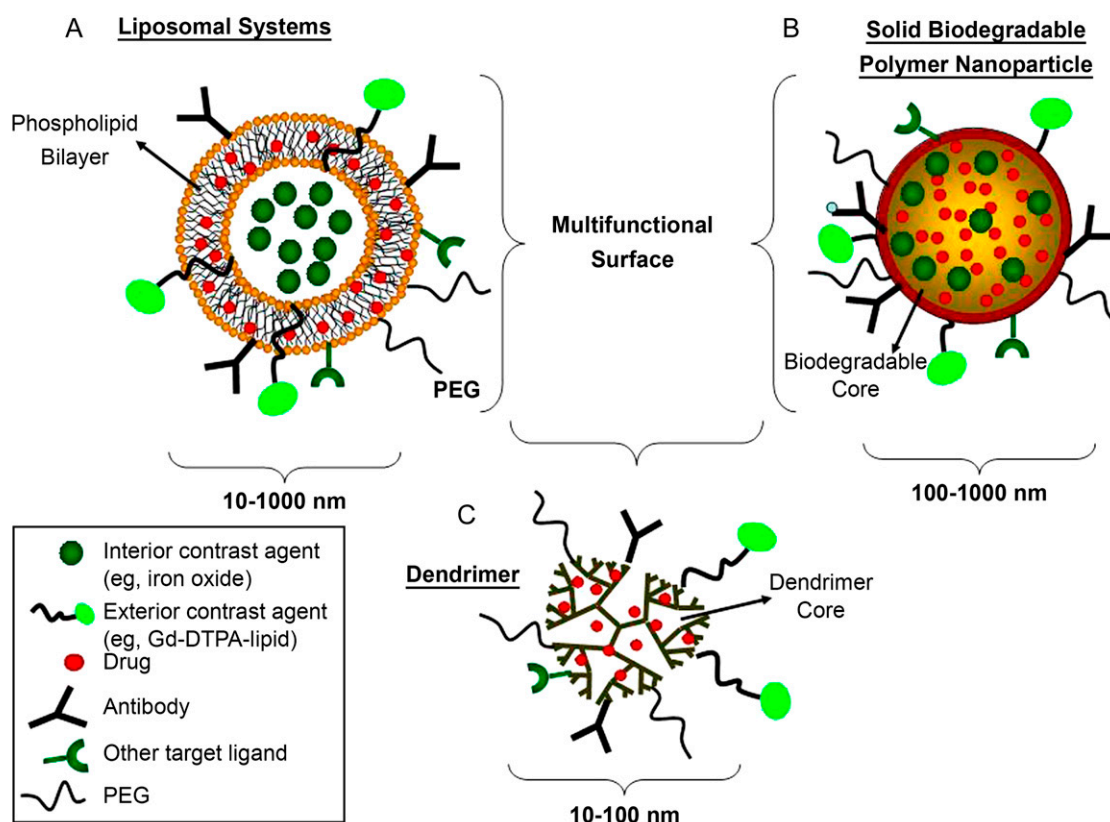

Figure 2. Schematic of nanosystems that may function as combined drug delivery and imaging agents for targeting T cells: (A) liposomal systems, (B) solid biodegradable nanoparticulates, and (C) macromolecular dendrimer complexes. PEG indicates polyethylene glycol; Gd-DTPA, gadolininum-diethylene triamine pentaacetic acid.

contrast in MR imaging. Paramagnetic reagents such as gadolinium-based chelates, manganese chloride, and iron oxide have been prepared in liposomal formulations and used to target different tissue types and cells.[42] Incorporation of the gadolinium-chelating lipids into the lipid bilayer during liposome formulation improves the relaxivity owing to enhanced water exchange with the surrounding environment and subsequent shortening of the relaxivity of the target of interest. Magnetoliposomes, which are liposomes that either incorporate iron oxide in the aqueous interior[54,55] or incorporate iron oxide nanoparticles coated with a lipid bilayer,[56] are effective T2 contrast agents that can be potentially engineered to release drug on exposure to a high-frequency magnetic field. These systems have been used for selective targeting and MR imaging of peripheral blood monocytes.[54]

It is the flexibility of engineering and construction that makes liposomal vehicles effective candidates for noninvasive imaging. As targeted drug delivery vehicles, some limitations of liposomes have emerged: most notably, poor control over release of the drug from the liposome (ie, the potential of leakage of drug into the blood), as well as questions regarding encapsulation efficiency, method scalability, and stability during storage.[57,58] Perhaps because of these limitations, the use of liposomes as combined contrast agents for T-cell tracking as well drug delivery has not been widely explored.

Macromolecular Systems: Dendrimers

Dendrimers are a class of monodisperse polymers distinguished by their repeated branching structure emanating from a central core.[59] This branching, which is inherent in the divergent synthesis of dendrimers, leads to a geometric growth of the polymer that can nearly approximate a sphere with increased branchings or higher generations (generation 6 or above). Additionally, the branching creates a labyrinthine core ideally suited for entrapment of a variety of small molecules such as drugs.[60,61] This fractal-like structure also creates a highly functionalized terminal surface suited for conjugation to ligands; this surface may facilitate targeting of the dendrimer or conjugation to contrast agents for imaging. As a result of these properties—and combined with their narrow molecular weight distribution and small size (less than 100 nm)—dendrimers have been used for a large number of applications, including drug delivery and contrast enhancement in MR applications.[62-64]

Polyamidoamine (PAMAM) dendrimers, synthesized by the repetitive addition of branching units to an amine core such as an ammonia or ethylene diamine, are widely used for drug delivery and imaging applications. PAMAM cores can function as drug reservoirs and have been studied as vehicles for small drugs,[60,65-67] paramagnetic molecules for contrast enhancement in MR imaging,[63] oligonucleotides,[68] transgenes,[69,70] and radionuclides.[71] Recently, the combined use of paramagnetic chelates and fluorescent groups conjugated to the surface of PAMAM dendrimers have facilitated dual-mode imaging of lymph nodes by both MR imaging and fluorescence microscopy.[72,73]

Because of the high number of functional groups on the termini combined with their potential for effective drug delivery, dendrimers have been exploited for targeting and visualization of a variety of tissue and cell types: by conjugation to folic acid for targeting tumors,[74] prostate-antigen-specific antibodies for targeting the prostate,[75] and peptides for targeting vascular endothelium[76] and intestinal epithelium.[77]

One strategy for constructing T-cell binding dendrimers couples streptavidin to the termini of PEG chains tethered to the dendrimer core. In this strategy, streptavidin is used to facilitate the coupling of biotinylated T-cell markers such as biotinylated anti-CD3 or peptide/MHC complexes that target polyclonal T cells and antigen-specific T cells, respectively.[78] The PEG tethered to the core and terminated with a functional group facilitates flexibility of attached ligands and allows attached proteins to scan a few nanometers of surface area for attachment to cell surface receptors. This is critical for enhancing the avidity to T-cell targets. In addition, this modification provides for enhanced solubility, biocompatibility, lower immunogenicity, and desirable pharmacokinetics, while the main biological functions such as receptor recognition are maintained.[79-81]

Our work has examined the utility of dendrimers in delivering doxorubicin, a widely used antiproliferative agent, to proliferating T cells and antigen-specific T cells. We found that the small size of the dendrimers, which allows for their efficient internalization, coupled with their ability to deliver a large number of doxorubicin molecules to specific T cells, led to an efficient system for inhibiting the specific proliferation of both polyclonal and antigen-specific T cells.[78]

In addition to dendrimers' utility as drug delivery vehicles to specific T-cell populations, several studies have now established their efficacy as contrast agents in MR imaging.[78,82,83] Our work has shown the potential of using these reagents in tracking the distribution of antigen-specific T cells (Figure 3). In these studies we compared the distribution of targeted dendrimer contrast agent in a transgenic mouse (OT-1) that expressed the OT-1 T-cell receptor specific for the peptide/MHC complex (H-2K^b loaded with the ovalbumin peptide) and in a negative control mouse with an identical genetic background lacking the OT-1 receptor transgene. These experiments involved injection of small quantities of gadolininum-diethylene triamine pentaacetic acid (Gd-DTPA)-pegylated PAMAM conjugated to the peptide/MHC complex via the tail vein of wild-type and transgenic mice; 3-dimensional MR images were acquired between 0 and 30 minutes after injection and posteuthanasia.

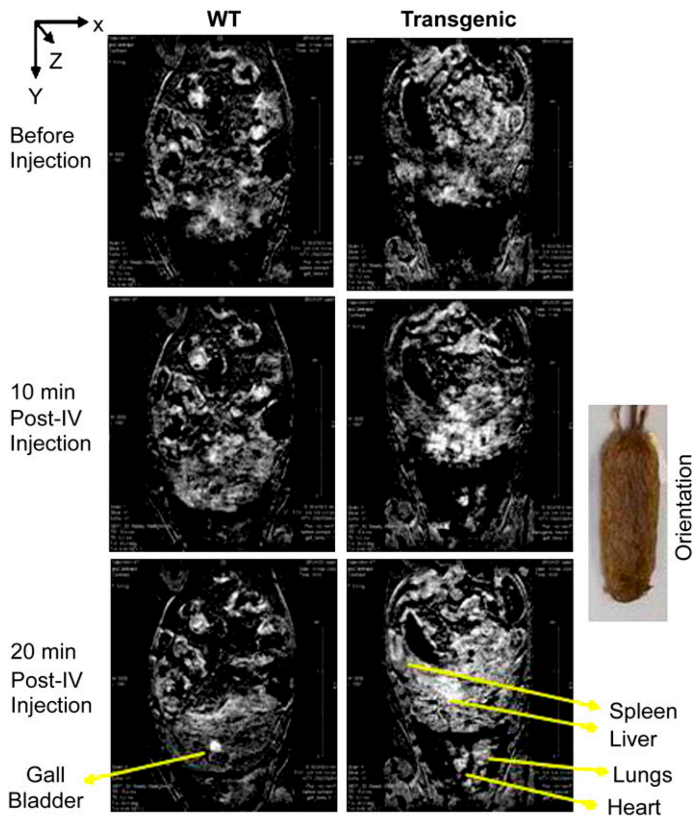

Figure 3. Antigen-specific T-cell distribution in WT and transgenic mice expressing the T-cell receptor specific for the peptide/MHC complex coupled to a polyamidoamine contrast agent. Magnetic resonance imaging of transgenic T cells in mice. T1 contrast enhancement in OT-1 transgenic mice at Z = –4.2 mm. T1-weighted fast gradient echo images are 512 × 256 × 64, relaxation time = 16 ms/echo time = 5.2 ms, 1-mm slice. Coronal images of a section of the mouse body at different time points after injection of peptide/MHC particulate contrast agent. The transgenic mouse showed significant brightening and motility of contrast postinjection compared with the WT control, which showed biliary accumulation (gall bladder) and excretion of contrast. WT indicates wild type; MHC, major histocompatibility complex.

Interestingly, the reagent showed longevity (up to 30 minutes and postmortem) in the heart and lungs of the transgenic mouse, while it was quickly cleared by biliary excretion (gall bladder uptake) from the wild-type mouse of identical background. This enhanced residence time and distribution were verified immediately after acquisition by fluorescence measurements of blood pools and of splenocytes dissected from both mice after euthanasia. The results also show that, consistent with previous data obtained by histological analysis,[20] antigen-experienced T cells may reside in and traffic heavily in nonlymphoid tissues. Perhaps the most encouraging aspect of these data is that the same construct was effective in noninvasive imaging and drug delivery to antigen-specific T cells, highlighting the promise of these systems for both functions.

Solid Biodegradable Nanoparticles

Solid biodegradable nanoparticulates fabricated from natural constituents such as proteins or lipids or synthetic polymers have been widely studied for targeted drug delivery to different types of cells. Emerging applications are now beginning to use some of these systems for imaging purposes. The main classes of these systems are based on either natural systems such as proteins conjugated to MR contrast agents or artificial polymers incorporating paramagnetic agents. Protein systems such as albumin-Gd-DTPA, while now available commercially, have had drawbacks such as limitations in routine and consistent synthesis of agents that possess similar chemical characteristics. In addition, the conformational changes, molecular shape, and size are difficult to predict or control. Albumin and other polypeptides are also limited in their drug delivery potential since only a few sites are available for conjugation to drugs and the potential for sustained drug delivery is low.

We focus here on one of the most widely used types of synthetic polymers, which are the aliphatic polyesters, specifically the hydrophobic poly(lactic acid) (PLA), the more hydrophilic poly(glycolic acid) (PGA), and their copolymer, poly(lactide-co-glycolide) (PLGA). These are attractive drug delivery vehicles because the degradation rate of these polymers, and the corresponding drug release rate, can vary from days (PGA) to months (PLA) and are easily manipulated by varying the ratio of PLA to PGA.[84] The drug can be encapsulated by a wide range of techniques, ranging from hydrophobic entrapment to conjugation to the polymer, providing further versatility in dosing. Moreover, the physiologic compatibility of PLGA and its homopolymers PGA and PLA have been established for safe use in humans; these materials have been used for more than 30 years in various human clinical applications, including drug delivery systems.[85,86]

For in vivo drug delivery to lymphocytes, the delivery of immunosuppressives from biodegradable nanoparticles can improve drug efficacy over that of free, unencapsulated drug for a variety of reasons.[70,87] One mechanism may simply be the sustained release of drug from localized drug reservoirs; particles that are not cleared away and release low levels of drug over a long time period have been shown to be more effective than bolus doses, presumably because even with localized bolus injections a higher percentage of free drug is cleared before any effect is exerted. In the case of nanoparticles, which are capable of being internalized by lymphocytes because of their small size, internalized PLGA nanoparticles can act as intracellular drug reservoirs, leading to continued exposure to and action of drugs over a sustained period of time. As expected, preliminary work has shown that encapsulation in nanoparticles can lower the IC_{50} of doxorubicin with a variety of cell lines and even increase

efficacy in cells displaying multidrug resistance.[70,88] As further evidence of the benefits of controlled release from nanoparticles, we have shown that particles improve the biological activity of doxorubicin to target T cells (Figure 4).

Targeting and controlled release would be an ideal combination for drug delivery, as the benefits of sustained and controlled release could be maximally exploited to increase intracellular drug concentrations, improve delivery to intracellular sites of drug activity, and bypass certain mechanisms of drug resistance. Control of drug release from internalized or localized PLGA nanoparticles is of further interest, as the benefits of sustained and controlled release can be used to modulate the rate, duration, and amount of intracellular drug concentration. The importance of controlled release has been seen with targeted liposomes, whose lack of ability to control release significantly limits their performance. For example, a few groups have demonstrated that intracellular drug concentrations can be greatly increased with doxorubicin-loaded liposomes targeted to internalizing receptors. Goren et al note, however, that the in vitro efficacy of doxorubicin is not necessarily improved, despite high intracellular concentrations.[48] They hypothesized that the drug is released by the "popping" of liposomes precipitated in the cytosol, which negates their efficacy and suggests that the total amount of intracellular doxorubicin delivered and the rate at which it is delivered are important to consider when designing targeted delivery.

On the other hand, when release can be controlled, substantial improvements in effectiveness are possible. Targeted PLGA nanoparticles that provide a controlled release of doxorubicin to target T cells are more potent at killing cytotoxic T lymphocytes (Figure 4). Based on this and other evidence, we believe that the rate and extent of duration of

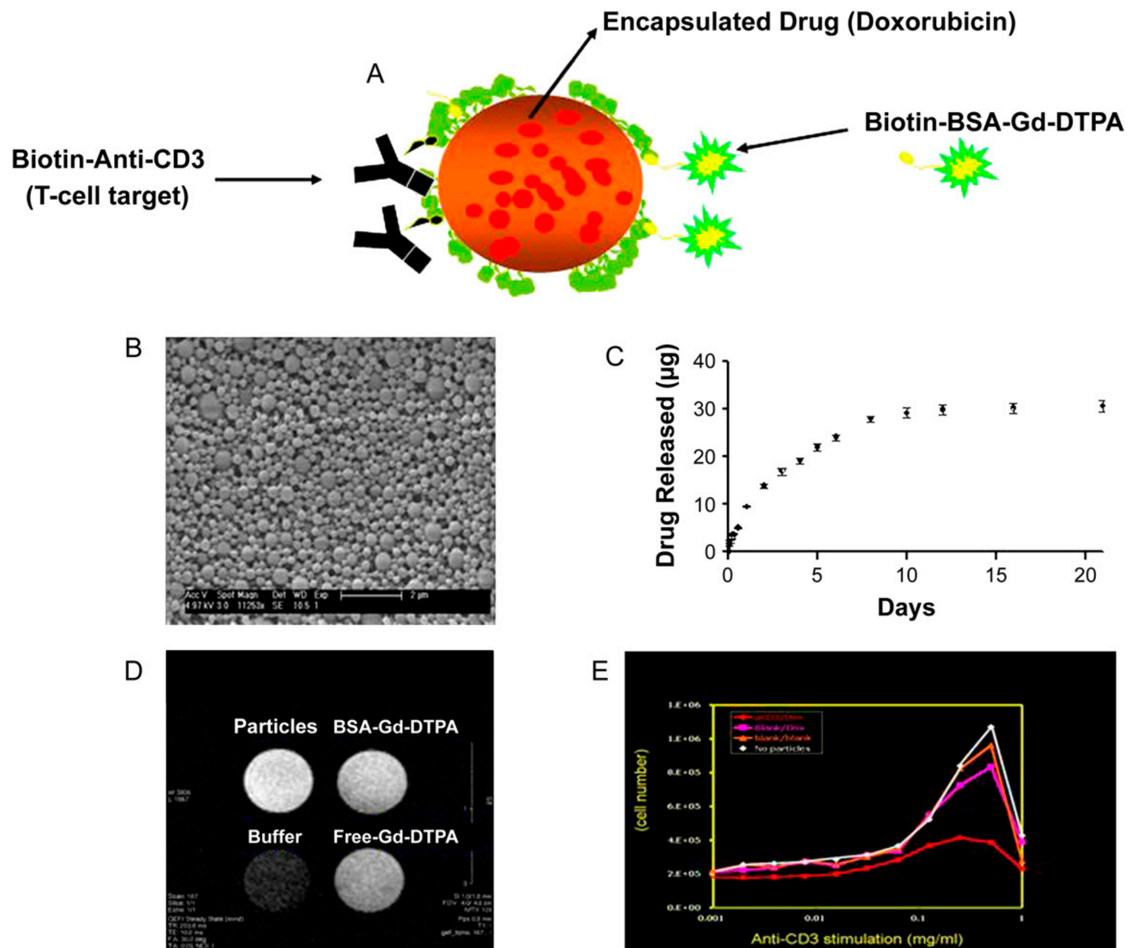

Figure 4. (A) Multifunctional solid biodegradable PLGA nanoparticles tethered to T-cell antibodies, magnetic resonance contrast agent (biotin-BSA-Gd-DTPA) and encapsulating an immunosuppressive drug (doxorubicin); (B) scanning electron microscopy image of PLGA nanoparticles; (C) cumulative release of doxorubicin from 10-mg nanoparticles under simulated physiological conditions (1 × phosphate-buffered saline, 37ºC); (D) T1-weighted image of contrast agent nanoparticulates; (E) the same nanoparticles are used for targeting and inhibition by doxorubicin delivery to polyclonal T cells stimulated in vitro with anti-CD3 (X-axis), cell number (Y-axis): red, targeted particles encapsulating doxorubicin; white, no particles; purple, doxorubicin particles (no targeting); orange, blank particles. CD3 indicates ; BSA, bovine serum albumin; Gd-DTPA, gadolininum-diethylene triamine pentaacetic acid; PLGA, poly(lactide-co-glycolide).

release from particles will be an important determinant of efficacy for T-cell drug delivery.

In addition to targeted drug delivery, recent work has demonstrated the viability of engineering different imaging modalities into biodegradable PLGA particles, rendering them detectable by fluorescence microscopy and MR imaging.[89] For example, recent work has shown that coencapsulation of Gd-DTPA with a fluorescent dye such as nile red in biodegradable PLGA particles enabled noninvasive monitoring of their local intravesical delivery with MR imaging for at least 5 days after the initial instillation, although the enhancement decreased because of microparticle degradation.[89] Our work has coupled T_1 contrast agents into PLGA nanosystems for visualization of the location of the drug dose (Figure 4). These systems use high-density surface modification with avidin-fatty acids that maintain a display of tethered ligands for prolonged periods, potentially enhancing contrast over long periods of time and overcoming issues related to the loss of imaging contrast with particle degradation.[78] PLGA systems are therefore clearly multifunctional and as such are promising candidates for simultaneous drug delivery and imaging to various types of cells and tissues. To our knowledge little has been done using these systems for targeting drug or MR contrast agents to lymphocytes.

CONCLUSIONS AND CHALLENGES

Most noninvasive tracking approaches for T cells in the body aim for isolation of those cells; ex vivo labeling with "molecular beacons" such as bioluminescent markers, PET, or MR contrast agents; and then injection of labeled cells into the whole body and analysis of their trafficking dynamics and localization. This information is important for diagnostic assessment of the many disease states that involve aberrant T-cell migration to tissue sites. Dextran-coated superparamagnetic iron oxide and other iron oxide–based nanoparticulates have emerged as leading candidates for this approach because of their high magnetic susceptibility coupled with the versatility in their coating with ligands that facilitate binding to target cells. This is a productive step forward in noninvasive cellular imaging; however, for tracking pathogenic lymphocytes combined with the potential for therapeutic intervention, other targeted nanosystems such as the ones discussed in this review offer considerable potential. These new capabilities may allow restoration of immune competence involving pathogenic lymphocytes and offer the potential for tracking the location and as well as localizing the drug depot to target T cells. Challenges in using these systems for routine monitoring and therapy include properly optimizing the surface to evade nonspecific endocytosis, understanding their clearance, and further evaluating their systemic toxicity and pharmacokinetics.

ACKNOWLEDGMENTS

T.M.F and W.M.S would like to express their gratitude for support from the Wallace Coulter Foundation and for National Institutes of Health Grant EB000487.

REFERENCES

1. Steinman L. Autoimmune-disease. *Sci Am*. 1993;269:106.

2. Hasler P. Biological therapies directed against cells in autoimmune disease. *Springer Semin Immunopathol*. 2006;27:443-456.

3. Ogg GS. T-cell immunotherapy of allergic disease: the role of CD8+ T cells. *Curr Opin Allergy Clin Immunol*. 2003;3:475-479.

4. Westermann J, Bode U. Distribution of activated T cells migrating through the body: a matter of life and death. *Immunol Today*. 1999;20:302-306.

5. Westermann J, Ehlers EM, Exton MS, Kaiser M, Bode U. Migration of naive, effector and memory T cells: implications for the regulation of immune responses. *Immunol Rev*. 2001;184:20-37.

6. Westermann J, Engelhardt B, Hoffmann JC. Migration of T cells in vivo: molecular mechanisms and clinical implications. *Ann Intern Med*. 2001;135:279-295.

7. Germain RN, Miller MJ, Dustin ML, Nussenzweig MC. Dynamic imaging of the immune system: progress, pitfalls and promise. *Nat Rev Immunol*. 2006;6:497-507.

8. Bradley LM. Migration and T-lymphocyte effector function. *Curr Opin Immunol*. 2003;15:343-348.

9. Hennecke J, Wiley DC. T cell receptor-MHC interactions up close. *Cell*. 2001;104:1-4.

10. Corr M, Slanetz AE, Boyd LF, et al. T cell receptor-MHC class I peptide interactions: affinity, kinetics, and specificity. *Science*. 1994;265:946-949.

11. Sykulev Y, Brunmark A, Jackson M, Cohen RJ, Peterson PA, Eisen HN. Kinetics and affinity of reactions between an antigen-specific T cell receptor and peptide-MHC complexes. *Immunity*. 1994;1:15-22.

12. Altman JD, Moss PA, Goulder PJ, et al. Phenotypic analysis of antigen-specific T lymphocytes. *Science*. 1996;274:94-96.

13. Constantin CM, Bonney EE, Altman JD, Strickland OL. Major histocompatibility complex (MHC) tetramer technology: an evaluation. *Biol Res Nurs*. 2002;4:115-127.

14. Howard MC, Spack EG, Choudhury K, Greten TF, Schneck JP. MHC-based diagnostics and therapeutics—clinical applications for disease-linked genes. *Immunol Today*. 1999;20:161-165.

15. Klenerman P, Cerundolo V, Dunbar PR. Tracking T cells with tetramers: new tales from new tools. *Nat Rev Immunol*. 2002;2:263-272.

16. Schwartz RH. T cell anergy. *Annu Rev Immunol*. 2003;21:305-334.

17. Casares S, Stan AC, Bona CA, Brumeanu TD. Antigen-specific downregulation of T cells by doxorubicin delivered through a recombinant MHC II–peptide chimera. *Nat Biotechnol*. 2001;19:142-147.

18. Cahalan MD, Parker I. Imaging the choreography of lymphocyte trafficking and the immune response. *Curr Opin Immunol*. 2006;18:476-482.

19. Iparraguirre A, Weninger W. Visualizing T cell migration in vivo. *Int Arch Allergy Immunol*. 2003;132:277-293.

20. Reinhardt RL, Jenkins MK. Whole-body analysis of T cell responses. *Curr Opin Immunol*. 2003;15:366-371.

21. Reinhardt RL, Khoruts A, Merica R, Zell T, Jenkins MK. Visualizing the generation of memory CD4 T cells in the whole body. *Nature*. 2001;410:101-105.

22. Cahalan MD, Parker I, Wei SH, Miller MJ. 2-photon tissue imaging: seeing the immune system in a fresh light. *Nat Rev Immunol*. 2002;2:872-880.

23. Wei SH, Miller MJ, Cahalan MD, Parker I. Two-photon imaging in intact lymphoid tissue. *Adv Exp Med Biol*. 2002;512:203-208.

24. Miller MJ, Wei SH, Cahalan MD, Parker I. Autonomous T cell trafficking examined in vivo with intravital two-photon microscopy. *Proc Natl Acad Sci USA*. 2003;100:2604-2609.

25. Osman S, Danpure HJ. The use of 2-[18F]fluoro-2-deoxy-D-glucose as a potential in vitro agent for labelling human granulocytes for clinical studies by positron emission tomography. *Int J Rad Appl Instrum B*. 1992;19:183-190.

26. Roddie ME, Peters AM, Danpure HJ, et al. Inflammation: imaging with Tc-99m HMPAO-labeled leukocytes. *Radiology*. 1988;166:767-772.

27. Edinger M, Cao YA, Hornig YS, et al. Advancing animal models of neoplasia through in vivo bioluminescence imaging. *Eur J Cancer*. 2002;38:2128-2136.

28. Mandl S, Schimmelpfennig C, Edinger M, Negrin RS, Contag CH. Understanding immune cell trafficking patterns via in vivo bioluminescence imaging. *J Cell Biochem*. 2002;87:239-248.

29. Costa GL, Sandora MR, Nakajima A, et al. Adoptive immunotherapy of experimental autoimmune encephalomyelitis via T cell delivery of the IL-12 p40 subunit. *J Immunol*. 2001;167:2379-2387.

30. Nakajima A, Seroogy CM, Sandora MR, et al. Antigen-specific T cell-mediated gene therapy in collagen-induced arthritis. *J Clin Invest*. 2001;107:1293-1301.

31. Herschman HR. Micro-PET imaging and small animal models of disease. *Curr Opin Immunol*. 2003;15:378-384.

32. Sosnovik D, Weissleder R. Magnetic resonance and fluorescence based molecular imaging technologies. *Prog Drug Res*. 2005;62:83-115.

33. Levchenko TS, Rammohan R, Volodina N, Torchilin VP. Tat peptide-mediated intracellular delivery of liposomes. *Methods Enzymol*. 2003;372:339-349.

34. Wunderbaldinger P, Josephson L, Weissleder R. Tat peptide directs enhanced clearance and hepatic permeability of magnetic nanoparticles. *Bioconjug Chem*. 2002;13:264-268.

35. Smirnov P, Lavergne E, Gazeau F, et al. In vivo cellular imaging of lymphocyte trafficking by MRI: a tumor model approach to cell-based anticancer therapy. *Magn Reson Med*. 2006;56:498-508.

36. Matuszewski L, Persigehl T, Wall A, et al. Cell tagging with clinically approved iron oxides: feasibility and effect of lipofection, particle size, and surface coating on labeling efficiency. *Radiology*. 2005;235:155-161.

37. Gregoriadis G, Neerunjun T. Homing of liposomes to target cells. *Biochem Biophys Res Commun*. 1975;65:537-544.

38. Gregoriadis G. Targeting of drugs: implications in medicine. *Lancet*. 1981;318:241-246.

39. Gregoriadis G. The carrier potential of liposomes in biology and medicine. *N Engl J Med*. 1976;704:110.

40. Gregoriadis G. Drug entrapment in liposomes. *FEBS Lett*. 1973;36:292-296.

41. Laverman P, Boerman OC, Oyen WJ, Dams ET, Storm G, Corstens FH. Liposomes for scintigraphic detection of infection and inflammation. *Adv Drug Deliv Rev*. 1999;37:225-235.

42. Mulder WJ, Strijkers GJ, van Tilborg GA, Griffioen AW, Nicolay K. Lipid-based nanoparticles for contrast-enhanced MRI and molecular imaging. *NMR Biomed*. 2006;19:142-164.

43. Torchilin VP. Liposomes as delivery agents for medical imaging. *Mol Med Today*. 1996;2:242-249.

44. Waterhouse DN, Madden TD, Cullis PR, Bally MB, Mayer LD, Webb MS. Preparation, characterization, and biological analysis of liposomal formulations of vincristine. *Methods Enzymol*. 2005;391:40-57.

45. Haran G, Cohen R, Bar LK, Barenholz Y. Transmembrane ammonium sulfate gradients in liposomes produce efficient and stable entrapment of amphipathic weak bases. *Biochim Biophys Acta*. 1993;1151:201-215.

46. Sapra P, Allen TM. Ligand-targeted liposomal anticancer drugs. *Prog Lipid Res*. 2003;42:439-462.

47. Allen TM, Sapra P, Moase E, Moreira J, Iden D. Adventures in targeting. *J Liposome Res*. 2002;12:5-12.

48. Goren D, Horowitz AT, Tzemach D, Tarshish M, Zalipsky S, Gabizon A. Nuclear delivery of doxorubicin via folate-targeted liposomes with bypass of multidrug-resistance efflux pump. *Clin Cancer Res*. 2000;6:1949-1957.

49. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov*. 2005;4:145-160.

50. Boerman OC, Laverman P, Oyen WJ, Corstens FH, Storm G. Radiolabeled liposomes for scintigraphic imaging. *Prog Lipid Res*. 2000;39:461-475.

51. Andreopoulos D, Kasi LP, Asimacopoulos PJ, et al. Selective in vitro labeling of white blood cells using 99mTc-labeled liposomes. *Nucl Med Biol*. 2002;29:185-190.

52. Harrington KJ, Mohammadtaghi S, Uster PS, et al. Effective targeting of solid tumors in patients with locally advanced cancers by radiolabeled pegylated liposomes. *Clin Cancer Res*. 2001;7:243-254.

53. Proffitt RT, Williams LE, Presant CA, et al. Tumor-imaging potential of liposomes loaded with In-111-NTA: biodistribution in mice. *J Nucl Med*. 1983;24:45-51.

54. Bulte JW, Ma LD, Magin RL, et al. Selective MR imaging of labeled human peripheral blood mononuclear cells by liposome mediated incorporation of dextran-magnetite particles. *Magn Reson Med*. 1993;29:32-37.

55. Martina MS, Fortin JP, Menager C, et al. Generation of superparamagnetic liposomes revealed as highly efficient MRI contrast agents for in vivo imaging. *J Am Chem Soc*. 2005;127:10676-10685.

56. De Cuyper M, Joniau M. Magnetoliposomes. Formation and structural characterization. *Eur Biophys J*. 1988;15:311-315.

57. Hans ML, Lowman AM. Biodegradable nanoparticles for drug delivery and targeting. *Curr Opin Solid State Mater Sci*. 2002;6:319-327.

58. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. *J Control Release*. 2001;70:1-20.

59. Wiener EC, Brechbiel MW, Brothers H, et al. Dendrimer-based metal chelates: a new class of magnetic resonance imaging contrast agents. *Magn Reson Med*. 1994;31:1-8.

60. Jansen JFGA, De Brabander-van den Berg EMM, Meijer EW. Encapsulation of guest molecules into a dendritic box. *Science*. 1994;266:1226-1229.

61. D'Emanuele A, Attwood D. Dendrimer-drug interactions. *Adv Drug Deliv Rev*. 2005;57:2147-2162.

62. Esfand R, Tomalia DA. Poly(amidoamine) (PAMAM) dendrimers: from biomimicry to drug delivery and biomedical applications. *Drug Discov Today*. 2001;6:427-436.

63. Kobayashi H, Jr, Kawamoto S, Jr, Jo SK, Jr, Bryant HL, Jr, Brechbiel MW, Star RA. Macromolecular MRI contrast agents with small dendrimers: pharmacokinetic differences between sizes and cores. *Bioconjug Chem*. 2003;14:388-394.

64. Klajnert B, Bryszewska M. Dendrimers: properties and applications. *Acta Biochim Pol*. 2001;48:199-208.

65. Liu MJ, Kono K, Frechet JMJ. Water-soluble dendrimer-poly(ethylene glycol) starlike conjugates as potential drug carriers. *J Polym Sci A*. 2000;37:3492-3503.

66. Kojima C, Kono K, Maruyama K, Takagishi T. Synthesis of polyamidoaminedendrimers having poly(ethyleneglycol) grafts and their ability to encapsulate anticaner drugs. *Bioconjugate Chem*. 2000;11:910-917.

67. Jansen JFGA, Meijer EW, de Brabander-van den Berg EMM. The dendritic box: shape-selective liberation of encapsulated guests. *J Am Chem Soc*. 1995;117:4417-4418.

68. Luo D, Haverstick K, Belcheva N, Han E, Saltzman WM. Poly(ethylene glycol)-conjugated PAMAM dendrimer for biocompatible, high-efficiency DNA delivery. *Macromolecules*. 2002;35:3456-3462.

69. Dennig J, Duncan E. Gene transfer into eukaryotic cells using activated polyamidoamine dendrimers. *J Biotechnol*. 2002;90:339-347.

70. Yoo HS, Lee KH, Oh JE, Park TG. In vitro and in vivo anti-tumor activities of nanoparticles based on doxorubicin-PLGA conjugates. *J Control Release*. 2000;68:419-431.

71. Kobayashi H, Wu C, Kim MK, Paik CH, Carrasquillo JA, Brechbiel MW. Evaluation of the in vivo biodistribution of indium-111 and yttrium-88 labeled dendrimer-1B4M-DTPA and its conjugation with anti-Tac monoclonal antibody. *Bioconjug Chem*. 1999;10:103-111.

72. Talanov VS, Regino CA, Kobayashi H, Bernardo M, Choyke PL, Brechbiel MW. Dendrimer-based nanoprobe for dual modality magnetic resonance and fluorescence imaging. *Nano Lett*. 2006;6:1459-1463.

73. Venditto VJ, Regino CA, Brechbiel MW. PAMAM dendrimer based macromolecules as improved contrast agents. *Mol Pharm*. 2005;2:302-311.

74. Pan D, Turner JL, Wooley KL. Folic acid-conjugated nanostructured materials designed for cancer cell targeting. *Chem Commun (Camb)*. 2003;19:2400-2401.

75. Daly T, Royal RE, Kershaw MH, et al. Recognition of human colon cancer by T cells transduced with a chimeric receptor gene. *Cancer Gene Ther*. 2000;7:284-291.

76. Sakharov DV, Jie AF, Filippov DV, Bekkers ME, van Boom JH, Rijken DC. Binding and retention of polycationic peptides and dendrimers in the vascular wall. *FEBS Lett*. 2003;537:6-10.

77. Wiwattanapatapee R, Lomlim L, Saramunee K. Dendrimers conjugates for colonic delivery of 5-aminosalicylic acid. *J Control Release*. 2003;88:1-9.

78. Fahmy T, Schneck J, Saltzman W. A nanoscopic multivalent antigen-presenting carrier for sensitive detection and drug delivery to T cells. *Nanomedicine.* In press.

79. Belcheva N, Baldwin SP, Saltzman WM. Synthesis and characterization of polymer-(multi)-peptide conjugates for control of specific cell aggregation. *J Biomater Sci Polym Ed*. 1998;9:207-226.

80. Belcheva N, Woodrow-Mumford K, Mahoney MJ, Saltzman WM. Synthesis and biological activity of polyethylene glycol-mouse nerve growth factor conjugate. *Bioconjug Chem*. 1999;10:932-937.

81. Katre NV. The conjugation of proteins with polyethylene glycol and other polymers altering properties of proteins to enhance their therapeutic potential. *Adv Drug Deliv Rev*. 1993;10:91-114.

82. Kobayashi H, Brechbiel MW. Dendrimer-based nanosized MRI contrast agents. *Curr Pharm Biotechnol*. 2004;5:539-549.

83. Kobayashi H, Brechbiel MW. Nano-sized MRI contrast agents with dendrimer cores. *Adv Drug Deliv Rev*. 2005;57:2271-2286.

84. Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv Drug Deliv Rev*. 1997;28:5-24.

85. Langer R, Folkman J. Polymers for the sustained release of proteins and other macromolecules. *Nature*. 1976;263:797-800.

86. Visscher GE, Robison RL, Maulding HV, Fong JW, Pearson JE, Argentieri GJ. Biodegradation of and tissue reaction to 50:50 poly(DL-lactide-co-glycolide) microcapsules. *J Biomed Mater Res*. 1985;19:349-365.

87. Emerich DF, Snodgrass P, Lafreniere D, et al. Sustained release chemotherapeutic microspheres provide superior efficacy over systemic therapy and local bolus infusions. *Pharm Res*. 2002;19:1052-1060.

88. Hu YP, Jarillon S, Dubernet C, Couvreur P, Robert J. On the mechanism of action of doxorubicin encapsulation in nanospheres for the reversal of multidrug resistance. *Cancer Chemother Pharmacol*. 1996;37:556-560.

89. Chen HH, Le Visage C, Qiu B, et al. MR imaging of biodegradable polymeric microparticles: a potential method of monitoring local drug delivery. *Magn Reson Med*. 2005;53:614-620.