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Self-Assembled Targeted Nanoparticles: Evolution of Technologies and Bench to Bedside Translation

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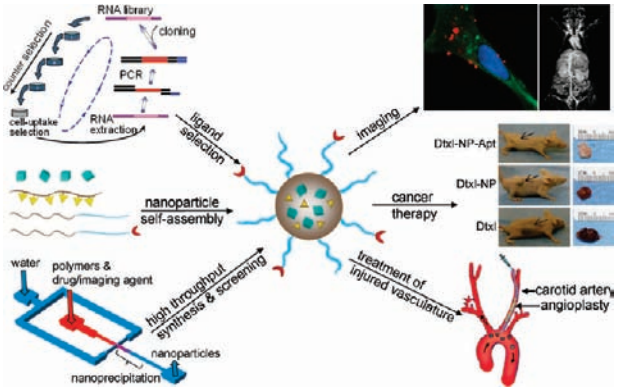

Nanoparticles (NPs) have become an important tool in many industries including healthcare. The use of NPs for drug delivery and imaging has introduced exciting opportunities for the improvement of disease diagnosis and treatment. Over the past two decades, several first-generation therapeutic NP products have entered the market. Despite the lack of controlled release and molecular targeting properties in these products, they improved the therapeutic benefit of clinically validated drugs by enhancing drug tolerability and/or efficacy. NP-based imaging agents have also improved the sensitivity and specificity of different diagnostic modalities. The introduction of controlled-release properties and targeting ligands toward the development of next-generation NPs should enable the development of safer and more effective therapeutic NPs and facilitate their application in theranostic nanomedicine. Targeted and controlled-release NPs can drastically alter the pharmacological characteristics of their payload, including their pharmacokinetic and, in some cases, their pharmacodynamic properties. As a result, these NPs can improve drug properties beyond what can be achieved through classic medicinal chemistry.

Despite their enormous potential, the translation of targeted NPs into clinical development has faced considerable challenges. One significant problem has been the difficulty in developing targeted NPs with optimal biophysicochemical properties while using robust processes that facilitate scale-up and manufacturing. Recently, efforts have focused on developing NPs through self-assembly or high-throughput processes to facilitate the development and screening of NPs with these distinct properties and the subsequent scale-up of their manufacture. We have also undertaken parallel efforts to integrate additional functionality within therapeutic and imaging NPs, including the ability to carry more than one payload, to respond to environmental triggers, and to provide real-time feedback.

In addition, novel targeting approaches are being developed to enhance the tissue-, cell-, or subcellular-specific delivery of NPs for a myriad of important diseases. These include the selection of internalizing ligands for enhanced receptor-mediated NP uptake and the development of extracellular targeting ligands for vascular tissue accumulation of NPs. In this Account, we primarily review the evolution of marketed NP technologies. We also recount our efforts in the design and optimization of NPs for medical applications, which formed the foundation for the clinical translation of the first-in-man targeted and controlled-release NPs (BIND-014) for cancer therapy.

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Introduction

The application of nanotechnology to medicine, also called nanomedicine, is expected to fundamentally change the landscape of pharmaceutical and biotechnology industries.[1−3] Tremendous investment in nanotechnology has been made by the United States government and other countries over the past 10 years. For example, the cumulative funding by the United States to create and support the National Nanotechnology Initiative (NNI) from 2001 to 2011 is $14 billion.[4] In parallel, most pharmaceutical companies have recently increased their focus in nanotechnology research. For example, Pfizer initiated the Addressable Drug Delivery via Engineered Particle Technologies (ADDEPT) Center of Excellence in 2010, and Johnson & Johnson established the Advanced Technologies and Regenerative Medicine (ATRM) subsidiary with a focus on regenerative medicine and nanotechnology. Despite this relatively new interest in nanotechnology, the first nanoparticle (NP) platform for medical applications, liposomes, dates back to the 1960s.[5] Over the past five decades, numerous other organic and inorganic NPs have been developed for disease diagnosis and therapy. Notably, with the maturation of nanoscience and together with increased financial support in this field, the pace of NP innovation is rising on a sharp slope. Figure 1 shows the number of publications per year based on the search terms "monoclonal antibody", "liposome", and "nanoparticle" in the last five decades. It is remarkable that while over the past decade the rise in the number of publications containing liposomes has been gradual, there has been a sharp increase in the number of NP publications, which mirrors that of the rapid rise for monoclonal antibody (mAb) publications in the 1980s. With the approval of Muromonab-CD3 in 1986 and over two dozen other biologics since then, mAbs represent a $50 billion market for pharmaceutical and biotechnology companies today.[6] We anticipate that the impact of NP technologies will surpass what we saw in mAbs for decades to come.

The clinically validated therapeutic and imaging NP products largely represent inefficiently targeted/non-targeted and relatively non-versatile systems, which only provide clinical benefit across a narrow range of therapeutic or imaging agents. The next-generation NP technologies are expected to overcome the aforementioned limitations of these first-generation NPs. Despite the enormous potential benefits of NPs for medical applications (Table S1 in the Supporting Information), however, the clinical translation of NP technologies has historically faced considerable

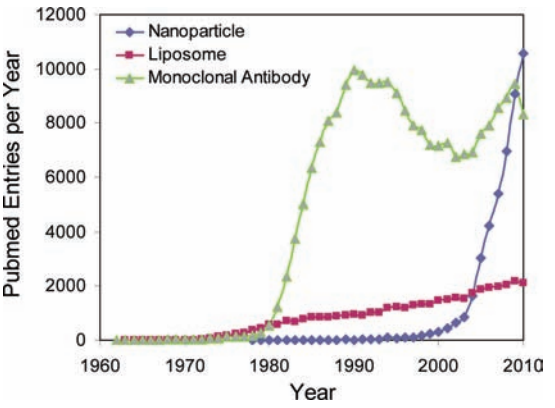

FIGURE 1. PubMed entries per year based on the search terms: "monoclonal antibody", "liposome", and "nanoparticle".

challenges. With the rapid emergence of clinically unvalidated novel nanomaterials and concurrent development of more complex NP technologies, the unforeseen toxicities, immune surveillance, and lack of scalability of these systems will undoubtedly hinder the development and commercialization of some of these technologies. While the use of clinically validated nanomaterials may lower the risk of translation and development of NP technologies, the toxicity of these systems will depend on multiple inter-related parameters including the biophysicochemical properties of NPs (composition, size, shape, rigidity, surface charge, hydrophilicity, and targeting ligands) and their payloads (drug/imaging agent type, solubility, loading, and release kinetics), and the accelerated blood clearance of some of these systems (e.g., PEGylated liposomes) could be potentially induced upon repeated dosing.[7] In addition, the limitations of current animal models (e.g., species-specific difference, insufficient recapitulation of human cancers and clinical response)[8] could also hinder the effective clinical translation of NP technologies. Thus, the successful development of therapeutic and/or imaging NPs will need to be optimized and evaluated on a case-by-case basis.

Evolution of Marketed Nanoparticle Technologies for Medical Applications

With advances in nanotechnology and our understanding of materials at the nanoscale, several distinct therapeutic NP platforms, including liposome, albumin NP, polymeric NP, and dendrimer, have been approved or entered clinical development for disease therapy (Figure 2). As for disease imaging, the iron oxide NP platform is thus far the only nanotechnology approved by the United States Food and Drug Administration (FDA).

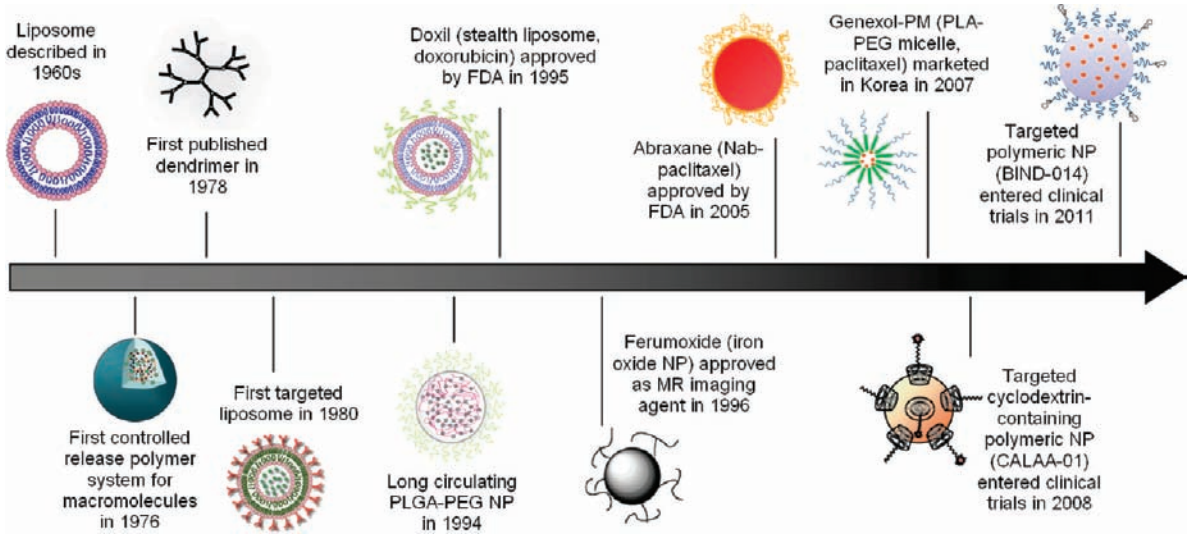

FIGURE 2. Historical timeline of clinical-stage nanoparticle technologies.

Liposomes became the first therapeutic nanomedicine to reach commercialization with the FDA approval of DOXIL (doxorubicin-liposome) in 1995. By encapsulating doxorubicin (Dox), the liposome technology changes the pharmacokinetics (PK) and biodistribution (BD) of Dox, allowing for longer circulation half-life and higher tumor concentration of this drug. While the maximum tolerated dose (MTD) of DOXIL (50 mg/m^2 every 4 weeks) was lower than that of standard Dox (60 mg/m^2 every 3 weeks) and DOXIL exhibited a new toxicity of hand-foot syndrome (palmar-plantar erythrodysesthesia), DOXIL enhanced the therapeutic index of Dox by reducing its cardiotoxicity and demonstrated efficacy in taxane-/platinum-resistant ovarian cancer.[9] Nevertheless, a major limitation of liposome technology is the lack of sustained release and the narrow range of chemical payloads that can be compatible with this platform.

With the FDA approval of Abraxane (Nab-paclitaxel) in 2005, nanoparticle albumin-bound (Nab) technology became the second class of therapeutic nanomedicine to be commercialized. When compared to standard paclitaxel (Taxol), Abraxane demonstrated significantly higher tumor response rates (33% vs 19%) and longer times to tumor progression (23.0 vs 16.9 weeks) among metastatic breast cancer patients who have failed combination therapy.[10] Different from Doxil, when Abraxane is reconstituted and injected into the bloodstream, albumin NPs rapidly disassociate into individual albumin molecules and then circulate with paclitaxel (Ptxl), thus minimally altering the circulation half-life and BD profiles of Ptxl.[11] Instead, the Nab technology significantly improved the MTD of Ptxl from 175 to 260 mg/m^2 every 3 weeks by enabling the exclusion of the toxic formulation excipient, Cremophor. Interestingly, when the Nab technology was used to deliver docetaxel (ABI-008; Nab-docetaxel), there was minimal difference between the MTD of ABI-008 and docetaxel (Dtxl), since Dtxl in its conventional form is formulated with tween that is relatively less toxic than Cremophor. Therefore, one limitation of the Nab technology is the relatively narrow range of validated compounds that can be reformulated through the elimination of toxic excipients and that could also bind to albumin.

Polymeric micelles were the third nanomedicine platform to be marketed, with the approval of Genexol-PM (Ptxl loaded polymeric micelles) in Korea in 2007. Similar to Abraxane, the polymeric micelle technology avoided the concomitant use of toxic Cremophor, and the MTD of Ptxl was increased to 300 mg/m^2 every 3 weeks for breast cancer treatment. Genexol-PM is currently in phase II clinical development in the United States.

Despite the success of these three NP systems in the clinic, their wide medical applications have been hindered due to the lack of sustained release and compatibility with diverse pharmaceutically active molecules (e.g., broad range of small molecules, proteins, and nucleic acids). On the other hand, controlled-release polymer technology has benefited virtually every branch of medicine since its first application in 1976.[12] Polymeric NPs can encapsulate various drugs and release them in a regulated manner via diffusion of the drug molecules through the polymer matrix or via differential surface and bulk erosion rates of the particles. By changing the polymer component, polymer molecular weight, and NP size, the drug release kinetics could also be efficiently tuned. Therefore, the polymeric NP technology could deliver drugs

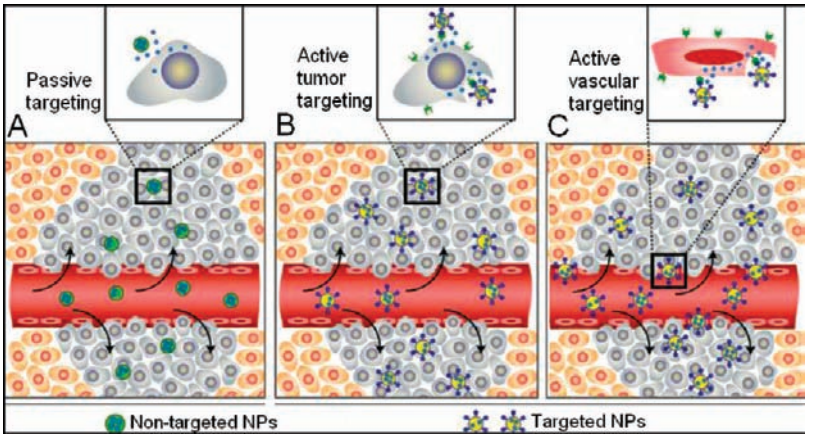

FIGURE 3. Passive vs active targeting. (A) Non-targeted NPs can passively extravasate through the leaky vasculature, which is characteristic of solid tumors and inflamed tissue, and preferentially accumulate through the EPR effect. (B) The presence of targeting ligands on the surface of NPs can result in active binding of NPs to cell surface antigens, leading to enhanced accumulation and cellular uptake through receptor-mediated endocytosis. (C) Targeted NPs are also critical for active vascular targeting, as the accumulation of NPs in the vascular wall is not a function of the EPR effect. Adapted with permission from ref 1. Copyright 2009 American Chemical Society.

at a sustained rate in the optimal range of drug concentration, thus enhancing the efficacy of drugs, maximizing patient compliance, and facilitating the use of highly toxic, poorly soluble, or relatively unstable drugs. By using targeting ligands against cell-surface antigens, targeted NP technologies could further lead to enhanced cellular uptake of NPs through receptor-mediated endocytosis, as compared to corresponding non-targeted NPs. The combination of targeted and controlled-release polymer NP technologies has recently resulted in the clinical translation of BIND-014 for cancer treatment.[13] It can be envisioned that, with the validation of NP products currently in clinical development, an increasing number of novel NP technologies (e.g., combination therapeutic NPs and theranostic NPs) will emerge for bench to bedside translation.

Targeted versus Non-targeted Nanoparticles

Impact of Non-targeted Nanoparticles. To date, 11 liposomal drugs and 1 Nab drug have been approved by the FDA for a myriad of clinical applications, along with one polymeric micelle product for oncologic use in Korea.[14] Three iron oxide NP products are also on the market for in vivo imaging use.[15] Interestingly, all of these products are non-targeted and relatively simple NP systems. In the case of cancer imaging and therapy, NPs mainly function by accumulating in tumor tissue through the enhanced permeability and retention (EPR) effect (Figure 3A),[16] which results from enhanced vascular permeability and the absence of a functioning lymphatic system. Therefore, for efficient NP accumulation, long circulation time is of critical importance and requires efficient particle evasion from the clearing organs including the liver, which is largely mediated by the physicochemical properties of the NPs.[1] Nevertheless, the EPR effect, which is highly dependent on the leaky tumor vasculature, could be limited in certain cancers (e.g., pancreatic cancer) with insufficient vascular permeability. In non-oncology diseases where vascular permeability is abundant, including inflammatory conditions, the EPR effect can also enhance the tissue accumulation of non-targeted NPs.

Targeted Nanoparticles: 30 Years in the Making. The first examples of cell-specific targeting using ligand-conjugated liposomes were described in 1980, and thereafter, a great number of targeted NPs were proposed and developed for drug delivery and imaging applications, with emphasis on cancer and cardiovascular diseases.[1,17] In the case of tumor cell targeting for diagnosis and therapy, the presence of targeting ligands can facilitate the retention and cellular uptake of NPs via receptor-mediated endocytosis (Figure 3B), although the tumor accumulation is largely determined by the particle physicochemical properties.[1] This is particularly essential for biomacromolecules (e.g., DNA and siRNA) that require intracellular delivery for bioactivity. As for vascular targeting for oncology or cardiovascular indications, ligand-mediated targeting will be critically important since NP localization is guided by ligand–receptor interactions rather than EPR (Figure 3C).

While the potential benefit of ligand-mediated targeting is clear, this technology has not made a significant clinical impact on human health. Within the 30 years since the first description of targeted liposomes, only three liposomal

TABLE 1. Ligand-Targeted Nanopartides in Clinical Trials

name	company	targeting ligand	receptor	platform	active pharmaceutical ingredient (API)	indication	status	ref
BIND-014	BIND Biosciences	peptide	PSMA	PLGA-PEG NP	docetaxel	solid tumors	phase I	13
CALAA-01	Calando Pharmaceuticals	transferrin	transferrin receptor	cyclodextrin-containing polymeric NP	siRNA	solid tumors	phase I	19
MBP-426	Mebiopharm Co., Ltd.	transferrin	transferrin receptor	liposome	oxaliplatin	gastric, esophageal, gastresophageal adenocarcinoma	phase Ib/II	20
MCC-465	National Cancer Center, Japan	$F(ab')_2$ fragment of human antibody GAH	N/A	liposome	doxorubicin	metastatic stomach cancer	phase I (not continued)	21
SGT53-01	SynerGene Therapeutics	single-chain antibody fragment	transferrin receptor	liposome	p53 gene	solid tumors	phase I	22

systems have made it to clinical trials (Table 1), among which MCC-465 does not appear to have progressed through clinical development after phase I completion and the other two are currently in early clinical trials. The reason targeted liposomes have demonstrated limited success in clinical development is complex and could be multifaceted. One major challenge may be attributed to the lack of robust and scalable methods in developing targeted liposomes with optimal biophysicochemical characteristics. On the other hand, with the recent development of self-assembly techniques using pre-functionalized polymers that have all of the desired NP components,[18] a targeted polymeric NP (BIND-014) has entered clinical trials at a rapid pace. More impressively, BIND-014 showed sustained drug release capability, without the need to increase manufacturing complexity. This self-assembly strategy enables the precise engineering of targeted NPs with distinct biophysicochemical properties, thus simplifying the optimization of these NPs. For example, BIND-014 with unprecedented PK, BD, efficacy, and tolerability properties was identified from the in vitro and in vivo screening of a large library of NP formulations. We believe that, with advances in targeted NP engineering technologies and high throughput screening methods, it will be increasingly feasible to rapidly develop targeted NP candidates for clinical translation.

Targeted Nanoparticles for Therapeutic and Theranostic Applications

Ligand–Polymeric Nanoparticle Conjugation for Targeted Drug Delivery. To improve the therapeutic index of drugs, we have pioneered the development of aptamer (Apt)-conjugated polymeric NPs for targeted delivery and controlled release.[23,24] The targeted polymeric NPs were formulated by first co-precipitating drugs (e.g., Dtxl) and polymers (e.g., poly(lactide-*co*-glycolide)-poly(ethylene glycol) (PLGA-PEG)) and then surface functionalizing with A10 Apt, which binds to the extracellular domain of prostate specific membrane antigen (PSMA), by carbodiimide coupling chemistry (Figure 4).[24] Using prostate cancer (PCa) as a model, the Apt-targeted polymeric NPs demonstrated a 77-fold increase in binding to prostate LNCaP cells versus nontargeted polymeric NPs.[23] These targeted NPs also exhibited remarkable in vivo efficacy.[24,25] For example, Dtxl-encapsulated PLGA-PEG-Apt NPs showed 100% survival, compared with the survivability of 57% for non-targeted PLGA-PEG NPs and 14% for Dtxl alone, in a 109-day study with LNCaP xenograft nude mice (Figure 5).[24] Moreover, by

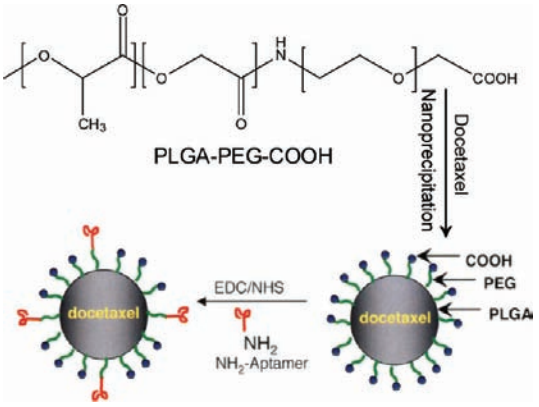

FIGURE 4. Schematic of the development of PLGA-PEG-Apt NPs. Adapted with permission from ref 24. Copyright 2006 National Academy of Sciences, USA.

encapsulating cisplatin prodrug, PLGA-PEG-Apt NPs displayed significant dose-sparing characteristics of cisplatin, with equivalent antitumor efficacy in LNCaP xenografts at 1/3 dose of cisplatin administered in its conventional form (0.3 vs 1 mg/kg).[25]

Ligand-Functionalized Polymers for Targeted Nanoparticle Self-Assembly. The conventional methods of synthesizing targeted NPs involve serial chemical processing of particles, whereby drug-encapsulated NPs are first formed, followed by the conjugation of targeting ligands. The post conjugation of targeting ligands requires the addition of an excess amount of reactants to ensure high coupling efficiencies, after which the ligand-conjugated NPs need to be further purified by removing the excess reactants. This

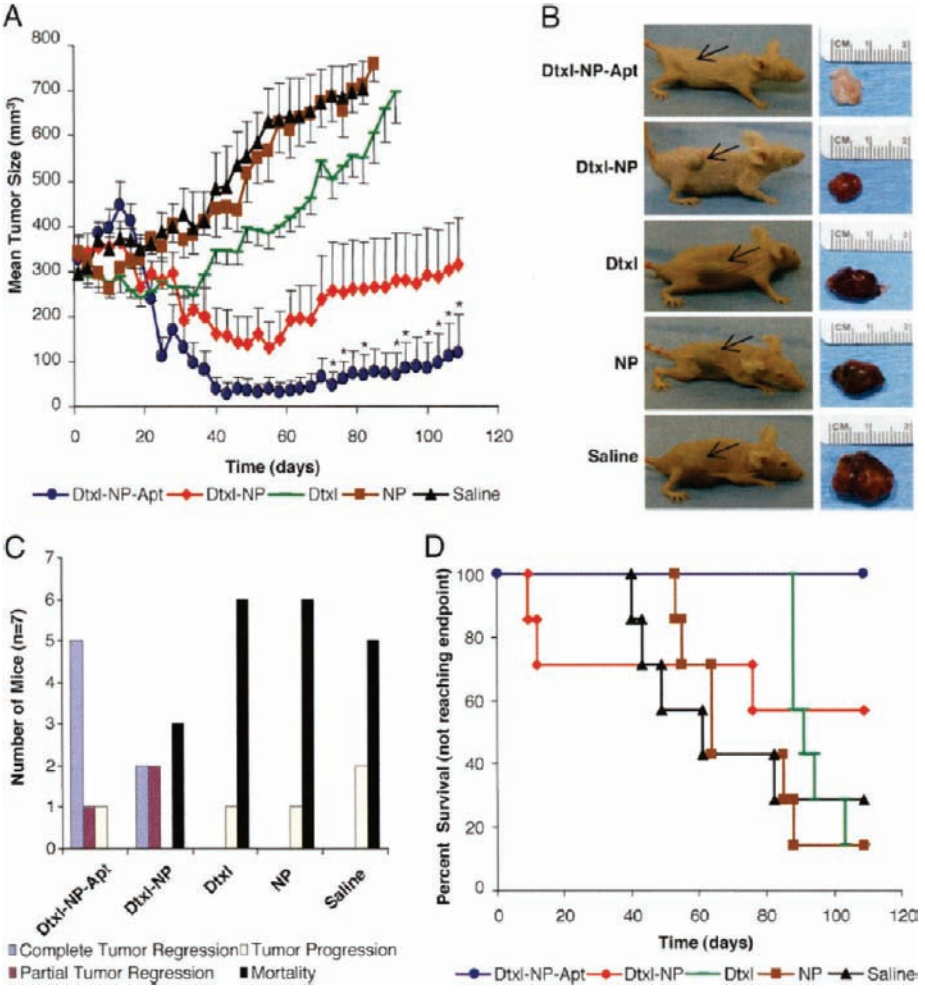

FIGURE 5. Comparative efficacy study in LNCaP xenograft nude mouse model. (A) The comparative efficacy study of saline, PLGA-PEG NP, Dtxl, Dtxl-NP, and Dtxl-NP-Apt over 109 days. (B) Representative mouse at end point for each group is shown (left) alongside images of excised tumors (right). (C) Plot of outcomes for each of the treatment groups divided into four categories: complete tumor regression, incomplete tumor regression, tumor growth, and mortality. (D) The Kaplan–Meier survival curve demonstrates that 100% of the Dtxl-NP-Apt group was alive on day 109. Adapted with permission from ref 24. Copyright 2006 National Academy of Sciences, USA.

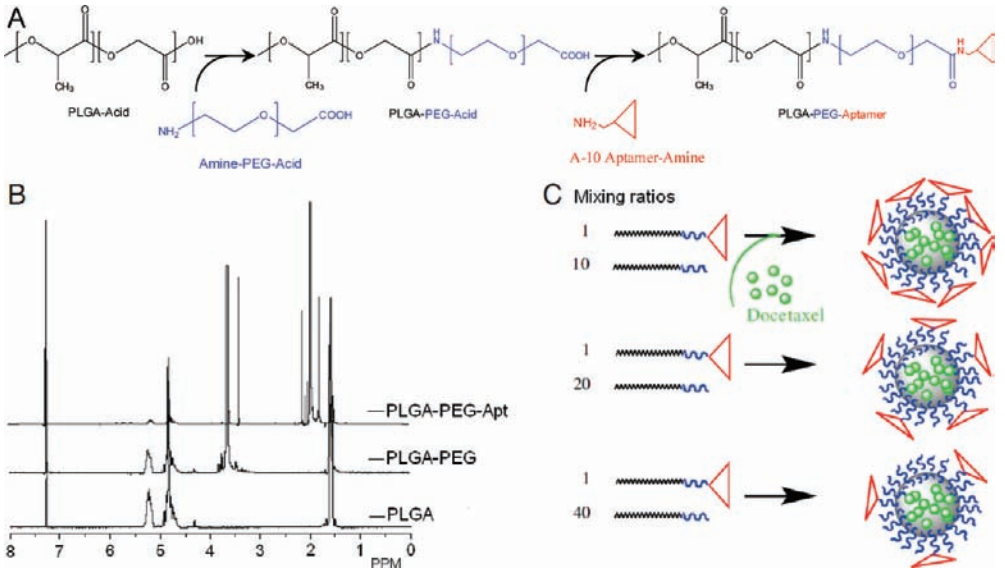

FIGURE 6. Development of self-assembled targeted NPs. The synthesis (A) and ^{1}H NMR characterization (B) of PLGA-PEG-Apt triblock polymer. (C) The self-assembly of PLGA-PEG-Apt NPs by nanoprecipitation. Using distinct ratios of PLGA-PEG-Apt and PLGA-PEG during NP formulation, the Apt surface density can be precisely and reproducibly changed. Adapted with permission from ref 18. Copyright 2008 National Academy of Sciences, USA.

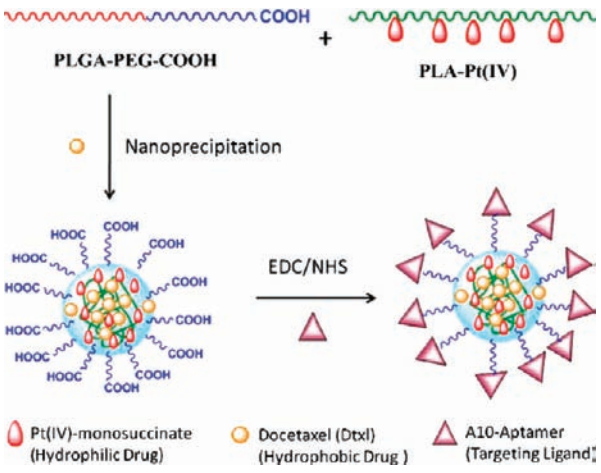

FIGURE 7. Design and construction of targeted NPs for the delivery of drug combinations. Adapted with permission from ref 26. Copyright 2010 National Academy of Sciences, USA.

added complexity makes it difficult to adjust the NP surface properties in a reproducible manner and the multistep processing contributes to unintended drug release from particles, resulting in unacceptable batch-to-batch variability in NP surface properties and drug load/release characteristics. To precisely engineer targeted NPs in a simple and scalable manner, an innovative strategy was developed by first prefunctionalizing polymer components with targeting ligands and then self-assembling with other NP components.[18] Figure 6 shows the development and characterization of PLGA-PEG-Apt triblock polymer and the self-assembly of targeted NPs simply by nanoprecipitating the mixture of PLGA-PEG, PLGA-PEG-Apt, and drug. By combinatorially varying the individual components, the NP biophysicochemical properties can be systemically and precisely changed, such as NP size, drug release kinetics, and differential targeting. Thus, this approach could eliminate the need for post-particle modification and enable the formulation of distinct targeted NPs with narrow variations for optimization. This technique has been successfully translated into the development of a targeted polymeric NP product candidate (BIND-014). Similarly, Tf-modified cyclodextrin-containing polymeric NPs (CALAA-01) were formulated by one-step self-assembly of four different components for targeted siRNA delivery in humans.[19]

Drug-Functionalized Polymers for Combinational Drug Therapy. Combination therapy by co-delivering multiple drugs via targeted polymeric NPs (Figure 7) was proposed to address the challenges that single-agent chemotherapy faces.[26] This strategy could provide several advantages, including (1) definitive delivery of a correct drug ratio to the target-of-interest for synergistic therapeutic effects, (2) suppression of drug resistance, and (3) control of each drug exposure in a temporal manner. As proof of concept, cisplatin and Dtxl were co-delivered to PCa cells with synergistic cytotoxicity by a targeted PLGA-PEG NP platform.[26] The hydrophilic Pt(IV) (cisplatin prodrug) was first conjugated to the polylactide derivative with pendant hydroxyl groups (PLA-OH) to yield a PLA-Pt(IV) polymer and then blended with PLGA-PEG and Dtxl during the nanoprecipitation process.

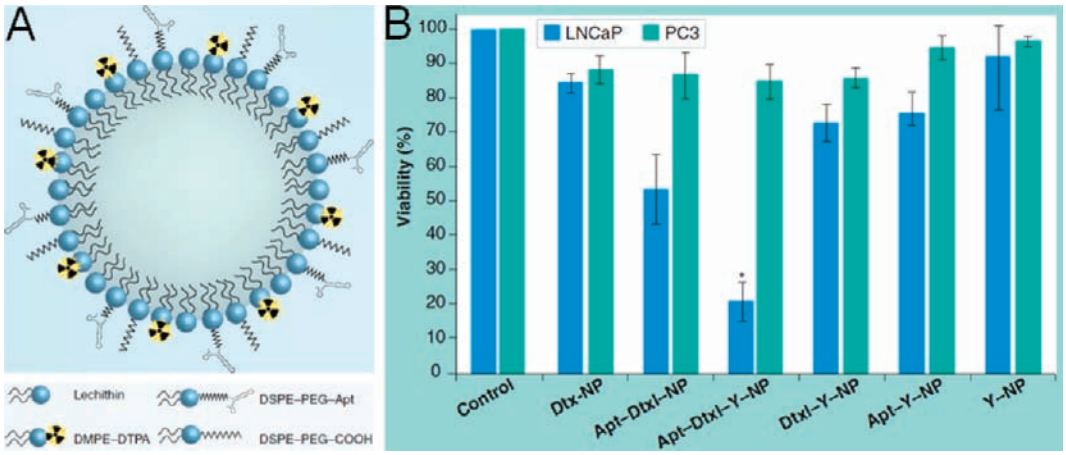

FIGURE 8. (A) Schematic of the ChemRad NP. (B) MTS cell viability assay of NPs. Adapted with permission from ref 27. Copyright 2010 Future Medicine Ltd.

The dual-drug encapsulated NPs were finally conjugated with the A10 Apt to develop a targeted co-delivery NP platform. In vitro studies demonstrate that the Apt-targeted, dual-drug encapsulated NPs are ~10 and 5.5 times more cytotoxic than PLA-Pt-NP-Apt and Dtxl-NP-Apt, respectively, suggesting their potential to deliver a synergistic combination of drugs for targeted cancer treatment. It is worth noting that, for combinational drug delivery applications, the release kinetics of each drug may have considerable effect on the efficacy of polymeric NPs, underscoring the need to fine-tune the stoichiometry of drugs as well as each drug's release kinetics for optimal in vivo efficacy and tolerability.

Lipid–Polymer Hybrid Nanoparticles for Chemoradiation Therapy. The development of chemoradiation (the concurrent administration of chemotherapy and radiotherapy) has led to significant improvements in local tumor control and survival. However, chemoradiation is limited by its higher toxicity, thereby precluding patients with poor general health from undergoing treatment. To improve efficacy and lower toxicity of this combination therapy, lipid–polymer hybrid NPs (Figure 8A) were developed for the co-delivery of chemotherapeutics and radiotherapeutics (ChemoRad NP).[27] The lipid–polymer hybrid NPs, which could potentially express the unique strengths of both liposomes and polymeric NPs while overcoming some of their limitations, were prepared by nanoprecipitation and self-assembly of PLGA polymers and biocompatible lipids.[28] Compared to PLGA-PEG NPs, the hybrid NPs present several advantages such as higher drug loading and slower drug release, which are mainly attributed to the existence of a lecithin monolayer at the interface of the PLGA core and PEG shell. For targeted co-delivery of chemotherapeutics (Dtxl) and radiotherapeutics (yttrium^{90}), the ChemRad NPs were engineered by self-assembling PLGA, lecithin, DSPE-PEG, DSPE-PEG-Apt, and DMPE-DTPA in a single step. The DMPE-DTPA monolayer can efficiently chelate with radioisotopes, while the PLGA core can carry Dtxl with a high loading efficiency. The targeted ChemoRad NPs showed much higher therapeutic efficacy than respective chemotherapy and radiotherapy treatment (Figure 8B),[27] suggesting that these NPs have the potential to be translated to clinical practice and to improve chemoradiation therapy.

Theranostic Nanoparticles by Combined Imaging and Therapy. Although in its infancy, theranostic NPs have shown potential in realizing personalized medicine by developing more effective and safer treatments specifically tailored for individual patients. Integrating molecular imaging and drug delivery, theranostic NPs can be used in diverse scenarios that range from improving disease diagnosis and therapy to better understanding various important aspects of the drug delivery process.[29] Many elegant studies have so far been carried out to demonstrate the theranostic principle by using targeted NPs.[30−33] For example, peptide-conjugated magnetic NPs have been developed to enable highly accurate magnetic resonance (MR) imaging, simultaneously with the delivery of therapeutics.[31]

We have recently evaluated the potential of the A10 Apt-conjugated thermally cross-linked superparamagnetic iron oxide NP (TCL-SPION-Apt) platform for targeted MR imaging and drug delivery.[32] Compared to the nontargeted TCL-SPION, the TCL-SPION-Apt led to a dramatic decrease in the longitudinal relaxation time T_1 and the transverse relaxation time T_2 for the LNCaP cells. Meanwhile, the TCL-SPION-Apt can carry Dox through absorption on the negatively charged NP surface and through

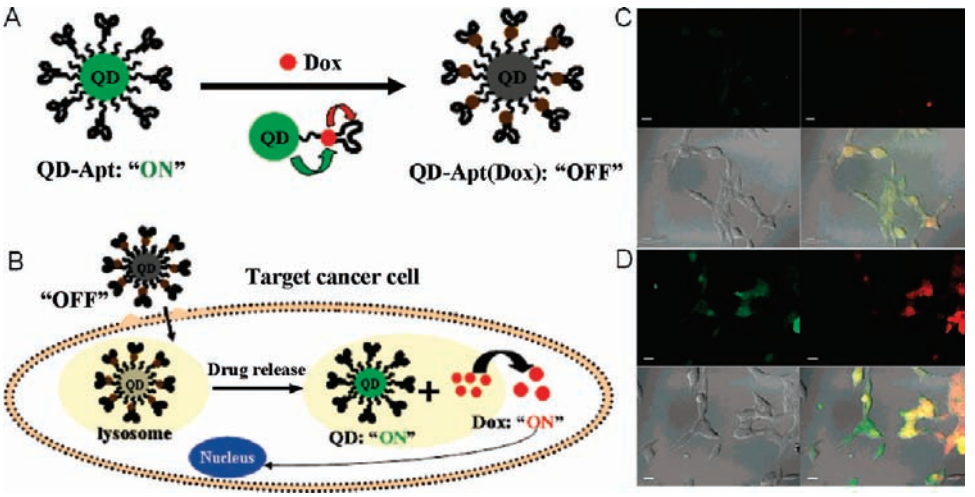

FIGURE 9. Schematic of QD-Dox-Apt Bi-FRET system (A) and receptor-mediated endocytosis of QD-Dox-Apt conjugates (B). LNCaP cells were imaged by confocal laser scanning microscopy after incubation with QD-Dox-Apt for 0.5 h at 37 °C, washed with PBS buffer, and further incubated at 37 °C for (C) 0 h and (D) 1.5 h. Adapted with permission from ref 33. Copyright 2007 American Chemical Society.

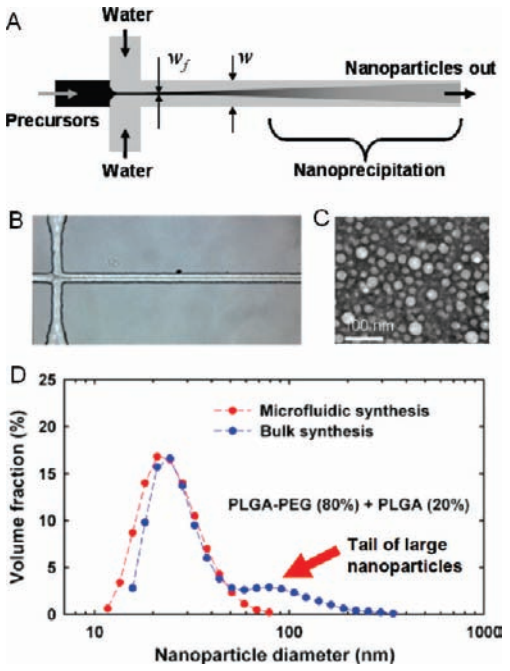

FIGURE 10. Microfluidic synthesis of PLGA-PEG NPs. (A) Schematic of on-chip synthesis by hydrodynamic flow focusing, with micrograph of device (B) and TEM image (C) of synthesized NPs. (D) Microfluidic synthesis enabled rapid mixing that improved homogeneity of the resulting NPs. Adapted with permission from ref 35. Copyright 2008 American Chemical Society.

intercalation with the double-stranded "GC" dinucleotide segment of the A10 Apt.

Along with disease diagnosis, it would also be ideal to understand the fundamental process of drug release after NP endocytosis, which could facilitate the rational design of targeted NPs for efficient drug delivery. To accomplish this goal, a smart CdSe/ZnS core–shell quantum dot (QD)-Dox-Apt system was engineered, capable of sensing drug release in a simple and easily detectable manner (Figure 9).[33] The fluorescence of both QD and Dox can be quenched by the intercalation of Dox within the A10 Apt ("OFF" state), through a bifluorescence resonance energy transfer (Bi-FRET) mechanism. Upon the specific uptake of QD-Dox-Apt conjugates into target cancer cells via receptor-mediated endocytosis, the release of Dox from the conjugates induces the recovery of fluorescence from both QD and Dox ("ON" state), thereby sensing the intracellular release of Dox and enabling the synchronous fluorescent localization and killing of cancer cells.

Microfluidic Platform for Nanoparticle Synthesis and Screening. One major consideration for the successful development of targeted NPs is the ability to identify the optimal NP biophysicochemical characteristics that could result in enhanced biodistribution and specific delivery. However, the screening of NP characteristics has been a challenge due to a complex interplay of physiological barriers with NPs, which is in turn affected in an interdependent manner by NP characteristics. For example, it is clear that particle size and surface properties play a major role in NP uptake by the mononuclear phagocyte system (MPS) cells in various organ systems.[34] Studying the effect of targeting ligand density has also revealed a relatively narrow window of ligand density that could result in the most favorable biodistribution of targeted NPs.[18] While a considerable amount of information has been learned regarding factors which affect NP biodistribution,[34] a high throughput system for systematically creating and screening targeted NPs with distinct biophysicochemical properties remains in great demand to accelerate the development of promising targeted NPs.

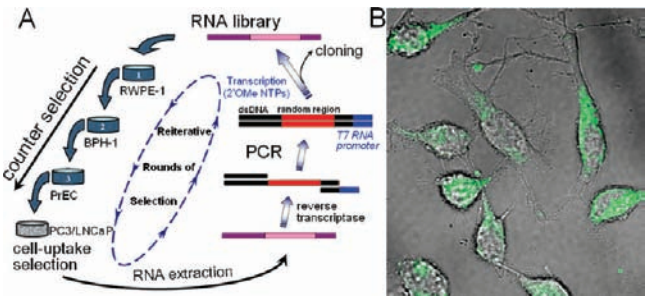

FIGURE 11. (A) Schematic of cell-uptake selection for enriching cell-specific internalizing Apts. (B) Confocal fluorescence image shows the internalization of Apt-targeted NPs to PC3 cells.

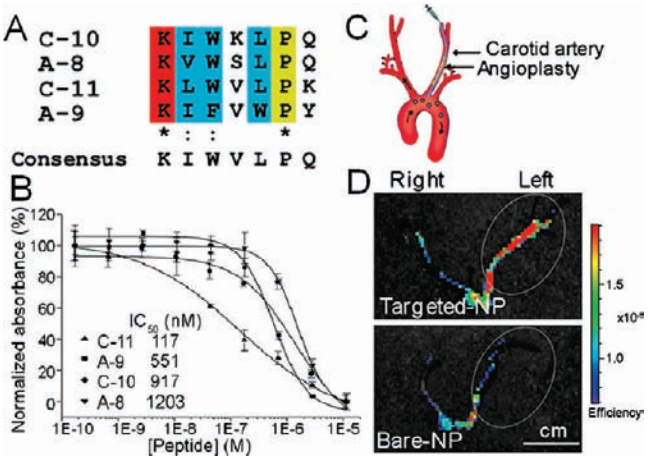

FIGURE 12. (A) Alignment and consensus sequence of phage clones. (B) Binding affinity of selected peptides. (C) In vivo intra-arterial delivery in a carotid injury model. (D) Fluorescence images overlaid on photographs of carotid arteries incubated with peptide-targeted NPs, compared with bare NPs. Adapted with permission from ref 39. Copyright 2010 National Academy of Sciences, USA.

We have recently developed a microfluidic technology that enables reproducible preparations of small and homogeneous PLGA-PEG NPs, hybrid lipid–polymer NPs, and lipid–QD NPs through rapid mixing (Figure 10).[35,36] By simply varying the flow rates, particle compositions, and precursor concentrations into the microfluidic device, the properties of the resulting NPs can be systematically and reproducibly controlled, which presents an opportunity to develop a high-throughput platform to rapidly synthesize libraries of distinct targeted NPs. Further in vitro screening by evaluating cell targeting, cytotoxicity, macrophage uptake, and immune response could lead to promising targeted NP candidates for in vivo PK, BD, and toxicity testing. Once the NP biophysicochemical parameters are identified, they can serve as a template to generate optimally engineered targeted NPs by larger scale synthesis for further scale-up.

Beyond NP synthesis, microfluidic systems can also be applied to optimize targeted NPs with high throughput capability. We used microfluidic channels lined with cells as a model of microcirculation to screen parameters that affect the interactions between targeted NPs and cells.[37] Compared to conventional in vitro screening methods that have lacked the control of the fluid flow, the development of such microfluidic systems, which are more representative of the biological microvasculature, could be of benefit in optimizing various parameters associated with NP–cell interactions. More comprehensive biomimetic microfluidic systems, such as "organ-on-a-chip",[38] could also be explored for the evaluation/optimization of targeted NP systems for therapeutic/theranostic applications.

Engineering of Targeted Nanoparticles against Complex Cellular Targets. Most of the targeted NPs currently under clinical and preclinical development are engineered by using ligands against simple cellular targets, such as well-characterized cancer antigens. However, the selection of targeting ligands is confounded by the limited number of cancer antigens that are sufficiently characterized for effective cancer targeting. Besides, only a subset of well-characterized antigens are taken up by cancer cells through membrane recycling pathways, which is necessary to enable the internalization of their associated ligands. Furthermore, given the commonly encountered intra- and intertumoral heterogeneous pattern of antigen expression, it may be advantageous to utilize a combination of ligands that collectively interact with multiple antigens on cancer cells. To achieve this goal, we have designed a cell-uptake selection strategy to enrich internalizing ligands (i.e., Apts) specifically against complex cellular targets, such as tumor cells (Figure 11A). In this strategy, Apts were isolated by using PCa cells as targets. Meanwhile, stringent counter selections were used to remove Apt candidates that interacted with non-targeted normal cells. More importantly, the cell-uptake selection was designed to enrich internalizing Apts rather than Apts with highest affinity as reported in previous selection processes, which may have bound to cells without internalization. Results demonstrated that the internalizing Apt-targeted polymeric NPs could be efficiently taken up by PCa cells (Figure 11B), and could drastically improve the cellular cytotoxicity of Dtxl, compared to non-targeted NPs.

Engineering of Targeted Nanoparticles against Extracellular Matrix. Considering that molecular targeting of cell-based targets may be confounded by inter/intrapatient heterogeneity in cell surface antigen expression, targeted NPs that can recognize the extracellular matrix have attracted considerable attention for therapeutic/diagnostic

delivery. We have recently engineered a peptide-conjugated NP to target the vascular basement membrane for the treatment of injured vasculature.[39] The high affinity C-11 peptide was screened from a combinatorial phage library of heptapeptide ligands against human collagen IV, which represents 50% of the vascular basement membrane (Figure 12). Angioplasty-injured carotid artery was used as a model of compromised vasculature to examine the targeting capacity of the C-11 peptide-conjugated polymeric NPs. The targeted NPs were delivered via both intra-arterial and i.v. administration and, when compared to non-targeted NPs, showed greater in vivo vascular retention at sites of injured vasculature in the rat (Figure 12D). Although the initial application was for vessel wall targeting in cardiovascular disease, the utility of this peptide-targeted NP system is broad and could be used to diagnose and treat different human diseases where the endothelial lining is compromised.

Conclusion

Nanoparticle technologies have already demonstrated significant impact on the fields of drug delivery and medical imaging. With the correct combination of a targeting ligand, an appropriate NP platform amenable to scale-up, a suitable drug/imaging agent, and a carefully selected disease indication, self-assembled targeted NPs can be developed for safer and more effective therapeutic or imaging applications. More complex targeted NP systems, which combine imaging and therapeutic agents or can trigger drug release at the target site when exposed to external stimuli (e.g., pH, ionic strength, enzyme, redox potential, temperature, light, ultrasound, magnetic field, and electric current), are also subject of ongoing research. These multifunctional NPs may also be useful in targeting circulating tumor cells, another important but still challenging area in cancer drug delivery. The "magic bullet" vision of Paul Ehrlich over 100 years ago is beginning to be realized, and with continued research and development efforts we expect targeted NPs to have a tremendous impact on human health for decades to come.

Supporting Information. Potential benefits of therapeutic and imaging nanoparticle technologies (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

BIOGRAPHICAL INFORMATION

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FOOTNOTES

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O.C.F. discloses his financial interest in BIND Biosciences and Selecta Biosciences, two biotechnology companies developing nanoparticle technologies for medical applications.

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