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Advances in the Clinical Translation of Nanotechnology

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Abstract

The use of novel materials in the nano-scale size range for applications in devices, drugs and diagnostic agents comes with a number of new opportunities, and also serious challenges to human applications. The larger size of particulate-based agents, as compared to traditional drugs, allows for the significant advantages of multivalency and multi-functionality. However, the human use of nanomaterials requires a thorough understanding of the biocompatibility of the synthetic molecules and their complex pharmacology. Possible toxicities created by the unusual properties of the nanoparticles are neither well-understood, nor predictable yet. A key to the successful use of the burgeoning field of nanomaterials as diagnostic and therapeutic agents will be to appropriately match the biophysical features of the particle to the disease system to be evaluated or treated.

Graphical abstract

Therapeutic RNAi wrapped around and protected by a carbon nanotube, adhered by high affinity electrostatic interactions, can be delivered to target cells. For details see (1).

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Introduction

The field of nanotechnology, which is the study and application of materials on the nanometer scale, is rapidly expanding its reach into many areas that will have great impact on computing, electronics and power, and healthcare. The nanomedicine field encompasses the development of new materials and their manipulation for devices, drugs and diagnostics. In this brief review, we will focus on recent translational advances in the use of nanometer-scale materials to develop diagnostic and therapeutic agents for human disease, focusing on drug and nucleic acid delivery, imaging, and sensors. Other comprehensive reviews of this enlarging field have been published (2–7).

Traditionally the materials that define this technology ranged from 1–100 nm in a single dimension. At this size range, particles may take on new bio-physical characteristics not revealed in the same materials at a larger or smaller scale. Importantly, it is also within this size range that most cellular machinery operates. This includes the organelles, motility machinery, and other protein and chromatin complexes within cells, the signaling pathways, the viruses that infect cells, and the secreted molecules that communicate with other cells, or cause their destruction.

The larger size of particulate-based agents, as compared to traditional drugs, allows for the significant advantages of multivalency and multifunctionality. Multivalency can confer both important changes in affinity and potency, which may lead to stronger signals at the cellular level and amplification of both diagnostic and therapeutic effects. Valency also causes differences in pharmacokinetics, such as longer off-rates at targets sites leading to prolongation of effects. Multivalency may additionally allow cross-linking of targets to achieve different effects than what would be observed with monovalent drugs. The enormous increases in avidity conferred by multivalency can also transform an ineffective signal into an effective one.

Multifunctionality, on the other hand, allows for the creation of more complex drugs that might be used simultaneously for both diagnosis and therapy within a single molecule. Such agents are known as “theranostics.” Appropriately designed multifunctional particles could

have components that allow selectivity to the target in vivo, tracing of its location in real time within the patient, and a therapeutic warhead effector.

With complexity and increased size, however, there are also potential complications that must be overcome. In particular, the systemic pharmacology of these new particles is still poorly understood (Figure 1). In part, this is due to the novel nature of many of the materials under study. For example, polymers, bearing repetitive charge motifs, or highly hydrophobic materials such as carbon nanotubes, will interact in unusual ways with cells within the body. This can have dramatic effects on their systematic clearance, retention in different regions of the body, and penetration into target sites. The sheer size of the particles alone, in the majority of cases, can reduce diffusion and extravascular access. Unusual pharmacokinetic properties as a result of the EPR effect (enhanced permeability and retention) must also be addressed.

Key components of the immune system and other cells or enzymes within the body, responsible for surveillance of the blood contents, such as endothelial cells in the liver, spleen, and marrow, along with nucleases, are often designed to clear particulate matter, perhaps as a defense against viruses, immune complexes, or damaged normal cells. Therefore, a key hurdle to the use of nanoparticle-based drugs has been understanding these rapid clearance mechanisms and manipulating them to advantage.

Nanoparticles for Medical Imaging

The use of contrast agents for diagnostic imaging enables non-invasive detection of pathologies. However, despite rapid technological advancements in medical imaging technologies, such as positron-emission tomography (PET), single-photon emission computed tomography (SPECT), and magnetic resonance imaging (MRI), the number of medical contrast media available to clinicians remains limited. Many contrast agents, enhance image contrast between tissue types, based mostly on different physical properties (mainly perfusion), but less frequently rely on disease-specific molecular signatures. Nanoparticles represent a promising and versatile new approach for the development of new and highly specialized contrast media (9).

With the exception of agents used in nuclear medicine, virtually all contrast media in current use passively accumulate as dictated by the physiochemical properties of the contrast medium; biodistribution of these agents is perturbed by factors associated with a particular disease. However, the diversity and flexibility of nanoparticles enables both passive and active targeting. For passive targeting of nanoparticles in cancer, the EPR effect is exploited (3). The EPR effect is responsible for the accumulation and retention of particles in the interstitial space due to the leaky nature of the tumor neovasculature and the lack of effective lymphatic drainage; the mechanics are similar to size exclusion chromatography. However, the EPR effect will be dependent upon the species (e.g., mouse versus human), particle size, and tumor type (10), among other factors. For active targeting, the biodistribution of a particle is specifically biased towards a particular tumor biomarker of interest, and can be achieved by functionalization with targeting moieties, such as small molecules, peptides,

aptamers, or antibodies. This enables the development of highly-specific molecular probes from particle-based contrast agents.

Investigational nanoparticles may incorporate a diverse array of molecular constructs (Figure 2). To provide image contrast, these particles may be functionalized with various imaging moieties. For example, nanoparticles may incorporate chelated gadolinium (MR contrast), isotopes (nuclear imaging), or fluorophores (optical imaging). For some nanoparticle platforms, contrast is an intrinsic property; for example, iron oxide nanoparticles (IONP) provide contrast in T2-weighted MR images, and bismuth particles add contrast in CT scans (11). Functionalization with yet an additional contrast moiety (e.g., appending an IONP with a PET radiotracer) allows for multimodal imaging, which could permit increased resolution and sensitivity in diagnostic procedures (9). Additionally, inclusion of β particle-emitting radiotracers allows for use of Cerenkov imaging, an investigational medical imaging modality, which can be utilized independently (via electron-emitting radionuclides) or multimodally with PET (via positron-emitting radionuclides). (12). By combining several imaging moieties into one particle, “smart” imaging agents have been created which are activated by interacting with their designated target (12), e.g., an enzyme cleaving the peptide bound between a nanoparticle and a fluorochrome, changing the emission signature and indicating the enzyme’s activity (13).

Since 1990, various IONP formulations have received regulatory approval as MR contrast agents; however, all but one of these products have been discontinued in the US due to limited adoption and competition from competing lower molecular weight contrast agents. Ferumoxytol (Feraheme®), an IONP approved for treatment of iron deficiency anemia, is currently undergoing several clinical trials for MRI-related applications (14). Silica particles, including “Cornell dots,” or C-dots, have been clinically evaluated for detection of metastatic melanoma (15). For ultrasound imaging, gas-containing microbubbles have been used clinically, but their usability was quite limited and complex. (16) In nuclear medicine, radiolabeled colloids have been used for many years to detect lymph nodes (17).

Nanoparticle constructs face high regulatory obstacles due to safety aspects, biodistribution, and biocompatibility in humans, as discussed above and reviewed in more detail in (8). The surface charge, shape, and diameter of nanoparticles are all important contributors to their ultimate distribution in the body (3) (Figure 2). Production challenges related to uniformity and batch-to-batch reproducibility must also be addressed for clinical applications.

In summary, nanoparticles have a high potential as diagnostic agents, as shown in multiple publications in preclinical settings. To date, however, for most new agents, potential as clinical diagnostic agents await data showing a clear advantage over current small molecules for molecular imaging; a notable exception is the intravascular contrast agents (8).

Overcoming hurdles to translating anti-cancer nanoparticles

There has been tremendous recent growth in the design of therapeutic nanoparticle delivery platforms, which have evolved to address pivotal challenges that have arisen from our understanding of cancer as a dynamic disease process. This evolution is abetted by the

unprecedented expansion of clinically-promising nanoscale systems for drug delivery (18), including polymeric particles, inorganic particles (i.e., silica (19)), metallic particles (i.e., gold (20)), iron oxide (20) and solid lipid-based materials, to address unmet clinical needs. Their tunable size, surface chemistry, and architecture confer distinct biological properties and enable transport of diverse payloads with high efficiency (21).

Clinically approved and translatable drug delivery systems have been designed to overcome a number of technical hurdles seen with early cancer nanomedicines that have limited their clinical benefit, such as unfavorable pharmacokinetics, dose-limiting toxicities, and narrow therapeutic indices(22). Liposomes are clinically available preparations, while other nanoformulated drugs, including polymeric nanoparticles and inorganic particles are at preclinical or early clinical stages of development. As the vast majority of these nanoformulated drugs are larger than ~10 nanometers (nm) in diameter, their successful non-specific delivery to tumors is thought to rely on EPR (3), which is typically accompanied by uptake in the reticuloendothelial system. Newer, targeted liposomal formulations are moving forward rapidly in clinical trials whereas polymeric particles, have had more limited progress, which may be linked to difficulties in formulation, characterization, and manufacturing(23). Few of the current drug delivery vehicles adapted with surface-targeting ligands, which should improve specificity, however, are in clinical trials, and none have been marketed. This may relate to chemical and pharmacokinetic complexities associated with the addition of targeting ligands, and concerns around synthesis, manufacturing, and costs. Whether the use of sub-10 nm diameter therapeutic particles can overcome key challenges of larger formulations is an area of active investigation (24).

The complexity of cancer cells, along with tumor heterogeneity and drug resistance, will likely make the use of single-agent molecularly targeted therapeutic platforms insufficient. To increase the effectiveness of cancer treatments, delivery strategies have progressively shifted towards combination approaches. Towards this end, substantial efforts have recently been directed towards developing (18) nanocarriers functionalized with multiple drugs that modulate and act through different pathways and diverse mechanisms (25) (19). Combination therapies incorporating biological therapeutics, such as genes, antibodies or siRNAs, in combination with other drugs(25, 26), or (20) "smart" nanocarriers, or stimuli-sensitive platforms(18, 25) may be used singly or in combination for active targeted cancer therapy. These approaches offer better spatial and temporal control of drug release in response to external cues or internal cues present in the tumor microenvironment. Internal cues triggering drug release include over-expressed enzymes, low pH, and elevated redox potential, while externally applied stimuli facilitating "on-demand" drug release include magnetic fields, ultrasound, and near-infrared light.

Upon exposure to such triggers, stimuli-responsive systems change in composition or conformation, and are accompanied by changes in physicochemical and/or drug release properties (25). A recent example of a translatable "smart" particle-based system exhibiting anti-cancer activity with internally-triggered cues involves an ultrasmall nanoparticle drug conjugate (NDC)(24), adapted from a clinically translated sub-10 nm fluorescent core-shell silica nanoparticle, C dots (15, 19). NDCs incorporate tyrosine kinase inhibitors, which are attached to the particle surface through a protease sensitive, self-immolative linker

responsive to lysosomal cysteine proteases (i.e., cathepsin B) for site-specific tumor therapy. Only a few “smart” platforms, responsive to external triggering, have recently made it to the clinical trial stage and/or have been approved for treating cancer(18, 20): thermosensitive liposomes (ThermoDox) and magnetic iron oxide particles (NanoTherm AS1, MagForce Nanotechnologies (20).

While, the design of nanocarriers sensitive to exogenous or endogenous stimuli, or their combination, may lead to improvements in drug delivery, which offer greater spatial and temporal control over the release of their cargoes relative to conventional delivery systems, these platforms are not completely devoid of off-target effects. Moreover, the use of nanotechnology in the co-delivery of multiple therapeutic agents, including molecularly targeted biologics and conventional small molecule therapeutics, is also receiving greater attention (27). While development of these combination drug-delivery systems is substantially more difficult than monotherapies, necessitating sophisticated optimization strategies, this should not require a significantly higher level of complexity in characterization, manufacturing, and quality control(24). Translation of these systems, however, will be challenging, and the cost-benefit ratio needs to be carefully considered. In the future, further integration of combination therapeutics with stimuli-sensitive nanodelivery systems may offer an exciting opportunity to maximize treatment benefit by extracting the best features of both approaches for specific cancer targets(25).

The strategic union of gene therapy and nanomaterials

Nanomaterials are uniquely positioned to assume a critical role in translating gene therapies into the clinic. RNA interference (RNAi) was introduced with high expectations for therapeutic application, (28) but has yet to overcome practical pharmacological obstacles related to tissue and cell specific delivery and untoward off-target effects following systemic administration. (29)The pharmacological goal of gene therapy is the delivery of bioactive polymeric deoxyribonucleic or ribonucleic acids (DNA or RNA) into a target cell to precisely alter the genome or transcriptome of the patient in order to treat disease. Delivery of DNA, RNA, messenger RNA, small interfering RNA, or microRNA frequently utilizes viral or non-viral vectors to transport nucleic acid cargoes. Nanomedicine has responded to Feynman’s challenge (30)producing a plethora of published studies and ideas, but too few clinical drug products have resulted. (7)

Designing biocompatible, non-viral, macromolecular platforms to mediate delivery of RNAi in order to optimize and improve tissue and cell-specific delivery in vivo has been a key concern. (1, 31–35)Virus-mediated delivery strategies have numerous short-comings that limit use in vivo. Non-viral systemic approaches have explored a diverse range of polymeric, organic, inorganic and biological platforms. Many of the later platforms are nanoscale macromolecules that are chemically suitable for gene delivery and advances in characterization of these materials have further promoted interest for nanomedical applications.

The first use of systemically administered siRNA in humans in 2010 used a nanoparticle delivery platform to target tumor and convey siRNA cargo. (32)Data from this phase I

clinical trial provided compelling evidence supporting a RNA interference mechanism in vivo and drug design success story. A growing number of clinical trials are underway employing novel material-mediated delivery of therapeutic RNAi (36).

The safe and effective systemic administration of nanomaterial-nucleic acid cargo faces several barriers to delivery in vivo (1). The nucleic acid loaded onto the nanoparticle must (i) not be degraded by serum endonucleases; (ii) avoid immune surveillance; (iii) not accumulate in tissues other than the desired target; (iv) extravasate from blood to tissue; (v) transit efficiently into the intended cell type; traffic to the appropriate intracellular compartment; and (vi) be readily released from the delivery platform in a bioactive form.

Revealing how the nanomaterial component of the drug interfaces with the biological milieu is crucial. Pharmacokinetic studies describe the distribution and excretion of a nanomaterial in vivo using an animal model. Further investigation can identify the cellular distribution in a tissue comprised of many cell-types. (1, 37) Elimination and off-target accumulation speak to toxicity issues. This approach was taken to develop a kidney proximal tubule targeting carbon nanomaterial that could deliver siRNA designed to prevent acute kidney injury. (24) The unexpected finding that synthetic carbon nanotubes are recognized by stabilin receptors expressed on the liver sinusoidal epithelium and mediate uptake of a synthetic molecular platform calls attention to the unique opportunity for designing cell-specific drugs. (37)

Nanomaterial based biologic sensors

Nanosensors enable the detection of analytes or biological phenomena via the transduction of a signal that is often facilitated by a unique property of the nanomaterial. For instance, many nanomaterials exhibit quantum confinement effects, which result in unique electronic or optical properties that enable transduction or amplification of binding events. Continued progress in the development of materials for the detection of bioanalytes has advanced several technologies to a level where clinical translation of nanosensors is foreseeable.

Multiple classes of technologies allowing rapid diagnostics and point-of-care detection are under development. Early clinical trials involving diagnostics which employ nanosensors include studies of volatile organic compounds (VOCs) in exhaled breath(38). Many of these devices are composed of field effect transistor-based sensors built from silica nanowires. Trials are completed or underway for the detection of biomarkers in lung cancer, multiple sclerosis, and persistent asthma(39).

Other nanotechnologies with potential for in vitro diagnostics have been commercialized, including NanoFlare sensors for the detection of mRNA in living cells, which has been applied for the detection of circulating tumor cells(40). This technology is based on gold nanoparticles bound to dye-conjugated antisense DNA, wherein the dye is quenched until it hybridizes to target mRNA and desorbs from the nanoparticle surface. Many other nanoparticle technologies under development for such assessments involve similar energy transfer and FRET mechanisms. Such devices have also demonstrated the possibility of triggering a therapeutic function, such as drug release, upon the detection of an analyte (41).

Several other nanoparticle-based nanosensors in development for use in vitro and in vivo involve surface enhanced Raman scattering (SERS). In these sensors, surface plasmons in metals such as silver or gold enhance the Raman scattering signal of an adsorbed material. Metal nanoparticles functionalized with molecular recognition elements such as antibodies or aptamers, enable measurements of analyses such as neurotoxins, glucose, and cancer biomarkers with the potential for detection down to zeptomolar levels (42).

Much excitement in the area of implantable and wearable sensors, for use in vivo, stems from “stretchable electronics”, which involve thin films often incorporating nanomaterials as sensor elements.(43) Such devices promise to collect spatially-defined mechanical, electrical, or chemical measurements from the skin, heart, or even the brain. Sensors for implantation in vivo have also been developed to emit near-infrared optical signals from within the body. Single-walled carbon nanotubes emit near-infrared light that can be detected up to centimeters through living tissues. Carbon nanotube fluorescence intensity and wavelength can be modulated via energy transfer events(44), changes in their dielectric environment, and electrostatic charge(45). A carbon nanotube-based sensor for nitric oxide was shown to function within the liver for up to 400 days.(46)

Several specific outstanding issues that impede the application and translation of sensor nanotechnologies include targeting efficiency and material toxicology. The efficiency in targeting probes to desired locations in the body and cell, including escape from the endolysosomal pathway, has recently been highlighted (47). Endosomal escape is often difficult to quantify, and improved understanding of material fate and transport mechanisms, such as the ‘proton sponge effect’ are needed (48). New comparative studies are needed to clarify and alleviate misconceptions regarding the acute and long-term toxicity of nanomaterial sensor components (49), (50),(51).

Conclusions

The use of novel materials in this size range comes with a number of new opportunities, and also serious challenges to human applications. These challenges have delayed widespread clinical adoption of nanometer-scale material-based drugs and diagnostics for all but a few examples (7, 52, 53). A key challenge for the use of nanomaterial-based drugs in humans is a better understanding of the biocompatibility of the synthetic molecules and their unusual pharmacology. Possible toxicities and immune responses (54) engendered by the distinct properties of the nanoparticles are neither well-understood nor predictable yet. In practice, nanomaterials range from extraordinarily inert polymers, such as carbon nanotubes (1, 37) to nucleic acids (55), to biodegradable carbohydrate-based materials (56). The size, shape, changes hydrophobicity, stiffness, and biodegradability will all have important consequences for the use of these drugs in vivo (57). In some cases, these properties can be used to great advantage by engineering new properties into the molecules to change the plasma half-life or site of organ delivery. Among others, excellent examples of these pharmacokinetic changes include the FDA approved lipid complex and liposomal drugs designed for cancers and infections. These agents have improved therapeutic indices because of better delivery of the same underlying toxic warhead to the target. The successful application of the burgeoning

field of nanomaterials as diagnostic and therapeutic agents will require an appropriate match of the biophysical features of the particle to the proposed disease under treatment.

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Highlights

- Particle size provides advantages of multivalency and multi-functionality, and new properties.
- Size, shape, charge, hydrophobicity, porosity, stiffness, and biodegradability are consequential.
- The systemic and cellular pharmacology of these new particles is still poorly understood.
- Nanomaterials will interact in unusual ways with cells within the body.
- Diagnostic nanoparticles can be adapted for use with MRI, PET, SPECT imaging modalities.

Figure 1.

Blood half-life, extravasation, renal clearance, uptake in the mononuclear phagocytic system (MPS), immunologic response, and flexibility to functionalize are important characteristics of imaging probes and theranostic compounds. These properties usually change depending on probe size. * = Strongly depends on surface properties of particles. ** = Option to increase detection sensitivity by attaching multiple imaging markers per molecule or strong endogenous imaging properties of the particle (eg, IONP's or microbubbles). Reprinted with permission from (8).

Figure 2. Schematic illustrations of nanomaterials platforms, which are approved, in preclinical development, or in clinical trials. © R. Bawa. Clinical Nanomedicine: Nanoparticles, Imaging, Therapy and Clinical Applications, Pan Stanford Publishing, Singapore. 2016