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Section III: Novel Nanomaterials for Diagnosis and Therapy

Mesoporous Silica Constructs

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Introduction

Specific drug delivery is one of the greatest challenges in cancer medicine. Targeted delivery of drugs encapsulated within nanocarriers can potentially ameliorate a number of problems exhibited by conventional 'free' drugs, including poor solubility, limited stability, rapid clearing, and, in particular, lack of selectivity, which results in non-specific toxicity to healthy cells and prevents the dose escalation necessary to eradicate diseased cells and overcome drug resistance. However, the physical and chemical properties of the nanocarrier, including size, shape, internal structure, and surface properties, play major roles in determining biodistribution of the carrier *in vivo*, biological interactions, cargo loading and release, biodegradation, and toxicity[1]. The optimal biodistribution and biological interactions of the nanocarrier can vary between different cancers (and individuals) making the ideal nanocarrier one in which the physical and chemical properties can be controlled and essentially tuned for the specific application[2]. An additional very necessary feature of an effective nanocarrier is the efficient loading and controlled release of the therapeutic cargos, which can range from small molecules to plasmids that have highly variable charge, polarity, and hydrophobic/hydrophilic character. Finally, a nanocarrier's potential to include imaging agents as well as drugs grants the possibility of creating 'theranostics', which allows both drug delivery and the monitoring of the course of therapy to be achieved with a single nanocarrier. In the context of creating a tunable nanocarrier, mesoporous silica nanoparticle constructs, developed over the past decade, have a distinctive *combination of features* that could enable their development as 'universal' nanocarrier platforms, of which, are simultaneously drug and disease agnostic.

Creation of Mesoporous Silica Nanoparticle Constructs

Mesoporous silica nanoparticles (MSNP) are composed of periodic arrangements or uniformly sized mesopores (ranging in diameter from 2 to >20-nm) embedded within an

amorphous silica framework and characterized by exceptionally high internal surface areas ranging from 500 to over 1200 m^2/g^3. MSNP are synthesized by two major routes: solution based synthesis or evaporation-induced self-assembly. Using solution based colloidal self-assembly it is possible to synthesize uniformly sized populations of MSNP with spherical, prismatic, torroidal, rod-like, or hollow shapes[4–8] with dimensions spanning 25-nm to over 250-nm, while in many cases maintaining low polydispersity indices <0.1[9]. Using evaporation induced self-assembly[10], it is possible to generate in a single step spherical MSNP with a predictable power law particle size distribution spanning 25-nm to over 250-nm. The highly tunable synthesis of MSNP allows for the selection of the size, size distribution, and shape most applicable based on the proposed delivery route and target biodistribution (**Figure 1A-D**).

During synthesis, the MSNPs can be modified to increase their functionality, for example their interiors can be constructed in a core/shell manner to introduce metal or metal oxide nanoparticles as imaging agents (**Figure 1E**). Core-shell MSNPs have seen many recent

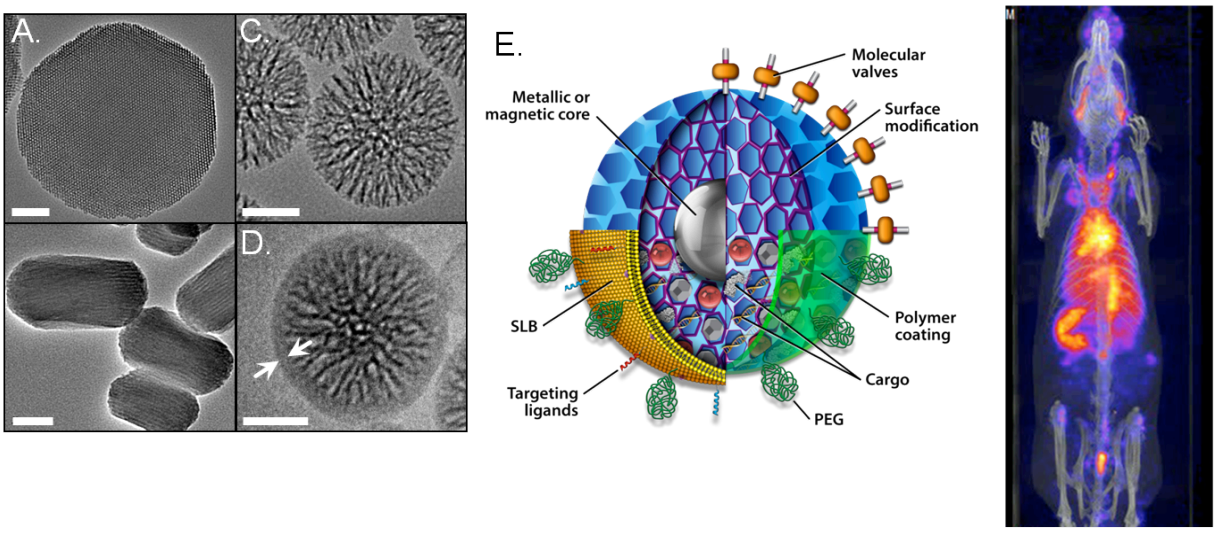

Figure 1. Mesoporous silica nanoparticles shape, pore size, lipid coating, functionalization and use. TEM images of spherical mesoporous silica nanoparticles with 2 nm pores (A), rod shaped mesoporous silica nanoparticles with 2 nm pores (B) and ~150 nm spherical mesoporous silica nanoparticles with 8 nm pores (C). CryoTEM of spherical mesoporous silica nanoparticles with 8 nm pores and a lipid bilayer coating highlighted by the white arrows (D). Scale Bars = 50nm. Schematic of a multifunctional mesoporous silica nanoparticle showing possible core/shell design, surface modifications and multiple types of cargo (E). SPECT image of radiolabeled 50nm mesoporous silica nanoparticles 5 hours post IV injection (F) (*Schematic (E) reprinted with permission from Tarn et al., 2013, TEM and SPECT images courtesy of Paul Durfee, University of New Mexico, Natalie Adolphi, University of New Mexico, and Yu-Shen Lin, Oncothyreon*).

applications in theranostics and allow for combined therapy and imaging simultaneously[11,12]. During or post-synthesis, the MSNP cores can also be loaded with fluorescent dyes with emissions spanning the visual range including; fluorescein isothiocyanate (FITC), rhodamine B isothiocyanate (RITC) and Cy3 as well as near-IR dyes such as AlexaFluor 700 and DayLight 680. The resulting MSNPs are extremely bright and optically stable enabling high-resolution multichannel optical imaging and quantitative multispectral flow cytometry. These labeled MSNPs provide a **unique** opportunity to examine the interaction between cells and nanocarriers along with MSNP biodistribution and delivery to tumors offering a direct measurement of these two important criteria during any regulatory approval[13,14].

Mesoporous Silica Nanoparticle Modification

MSNP functionality can be introduced by modifying silanol groups (ΞSi-OH) present both within the pore interiors and on the outer surface. Silanol groups are chemically accessible and can be easily reacted with alkoxy or chlorosilane derivatives to introduce organic functionality. Modification performed in single step or multi-step procedures provides an almost unlimited ability to 'tune' the charge, polarity, and hydrophobic/hydrophilic character of the pore and exterior particle surfaces, provide sites for further chemical conjugation or chelation with targeting and control ligands, and to couple imaging agents including radio labels for SPECT imaging (**Figure 1F**). Chemical moieties can also be adsorbed onto MSNP, especially facilitated by negatively charged SiO- groups, resulting from deprotonation of surface silanol groups at neutral pH, which result in attractive electrostatic interactions with positively charged moieties.

Introducing functional groups on the MSNP exterior surface gives rise to additional surface properties. They can be further reacted as linkers to attach larger molecules or used to adsorb coatings through noncovalent interactions. For the latter case, polymers are commonly employed on MSNPs[13,15,16]. Due to the intrinsic negative charge of the silica surface resulting from deprotonation of surface silanols, bare nanoparticles can be electrostatically functionalized with a positively charged polymer. Polymers or other surface bound functional groups can also be used to retain cargo within the MSNP and aid in colloidal stability that is required keep MSNPs highly dispersed for biomedical applications. An alternative means of surface coating MSNPs is by fusion with phospholipid bilayers to form a construct referred to as a *protocell*[14,17]. The cryo-TEM image (**Figure 1D**) shows a mesoporous silica particle core prepared by EISA enveloped by a conformal, 4-nm thick supported lipid bilayer (SLB). The properties of the SLB can be varied widely using lipids with differing fluidities or melting transition temperatures and headgroup chemistries that dictate charge and chemical reactivity. Membrane-bound components like cholesterol along with PEG can be introduced to control the fluidity and stability of the SLB, and it can be chemically

conjugated with ligands to effect targeting and internalization (*vide infra*) (**Figure 2**). As with polymer coatings, the SLB can serve to retain cargo introduced into the MSNP interior and aid in colloidal stability for biomedical applications. *Protocells* however have the advantage that acidification, as occurs in a tumor microenvironment or endosome, serves to permeabilize/destabilize the supported lipid bilayers triggering release of cargo[14,18].

Cargo Loading, Targeting and Cargo Delivery

Three major features of mesoporous silica constructs; high surface area, controllable pore size, and the ability to tune the charge of the particle, make them ideal for loading of varied cargo. Small molecule drugs and biological entities such plasmids or mRNA cargo present a large size range, which requires variable pore sizes for cargo loading. Using surfactants or block copolymers as structure directing agents in conjunction with swelling agents, it is possible to control pore size[19] from ~2-nm to over 20-nm, while hollow or toroidal particles provide even larger pore sizes (**Figure 1A-D**).

The tunable surface characteristics in combination with the high surface area allows for the simple loading of high concentrations of diverse classes and combinations of cargos that can be delivered by endocytosis or macropinocytosis[20]. The uniform arrangement, size, and connectivity of the porosity established by self-assembly confer to a MSNP very high BET (i.e., Brunauer–Emmett–Teller theory) surface areas ranging from 500 to over 1200 m^2/g. Surface area is important because it is the drug accessible surface area that dictates the drug loading capacity of an MSNP.

MSNPs can accumulate in tumor targets through both passive and active targeting. Passive targeting schemes rely on the enhanced permeability of tumor vasculature (the so-called enhanced permeability and retention (EPR) effect) to direct accumulation of nanocarriers at tumor sites, but the lack of cell-specific interactions needed to induce nanocarrier internalization decreases therapeutic efficacy and can result in drug expulsion and induction of multiple drug resistance (MDR). In terms of passive targeting, coating of MSNPs with a cationic polymer (e.g., PEI) significantly facilitates their uptake into tumor xenografts[16]. More recently, combining size control of MSNPs and PEI/PEG copolymer coating resulted in enhanced EPR effect in a xenograft tumor model[15].

To limit the degree of nonspecific binding while enhancing specific internalization by the target cell or tissue, MSNPs can be actively targeted toward an intended region (**Figure 2A**). Active targeting employs ligands that bind specifically to receptors overexpressed on the cancer cell surface. Bioactive ligands, such as folate, RGD peptide, and transferrin have been employed due to their respective receptors being overexpressed on many

different cancer cell types[21]. In general, high specificity and binding affinity require a high concentration of surface-conjugated ligands to promote multivalent binding effects, which results in more efficient drug delivery through receptor-mediated internalization pathways. However, high ligand densities can promote nonspecific interactions with endothelial and other noncancerous cells and increase immunogenicity, resulting in opsonization-mediated clearance of nanocarriers via the mononuclear phagocyte system (MPS). In this regard, the MSNP supported lipid bilayer construct (i.e., *protocell*) provides some potential advantages because its fluid SLB enables targeting ligand recruitment to target cell surface receptors, promoting high avidity with a low overall peptide concentration (**Figure 2B**).

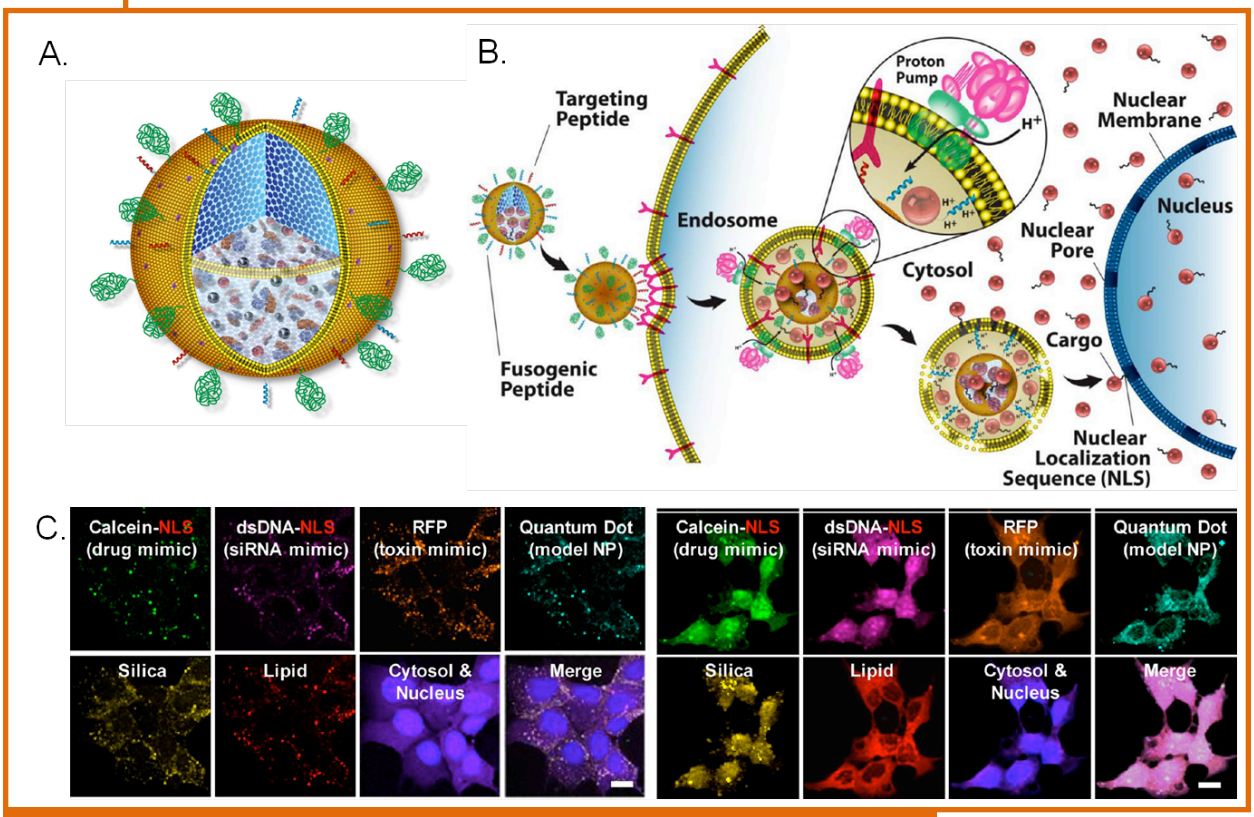

Figure 2. (A) Schematic of the protocell showing the MSNP core containing various cargo; such as drugs, nucleic acids and fluorophores, and coated with a lipid bilayer which has been functionalized by targeting ligands and PEG. (B) Schematic diagram depicting the successive steps of the multivalent binding and interanalization of targeted MSN –supported lipid bilayers, followed by endosomal escape and nuclear localization of MSNP-encapsulated cargo. (C) Hyperspectral confocal imaging of targeted delivery of multicomponent cargos in protocells to Hep3B cells for 15 minutes (left panel) or 12 hours (right panel) at 37°C. Alexa Fluor 532-labeled nanoporous silica cores (yellow) were loaded with calcein (green), an Alexa Fluor 647-labeled dsDNA oligonucleotide (magenta), RFP (orange), and CdSe/ZnS quantum dots (teal). Cargos were sealed in the cores by fusion of Texas Red-labeled DOPC liposomes (red) (*Reprinted with permission from Tarn et al., 2013*).

Thus, simultaneously with porosity, tunable surface and internal chemistry of the MSNP allowing for the inclusion of multiple cargos, MSNPs with lipid or polymer coating and cell type-specific targeting create a very robust single multifunctional nanocarrier platform (**Figure 2C**).

The highly tunable nature of MSNPs has also provided an ideal platform for the development of even more advanced nanocarriers with specific and controlled release of their cargo. The uniform pore size coupled with facile surface chemical conjugation has enabled modification of the pore entrances or interiors with responsive (light, pH, redox, etc.) molecular machines that can serve as gates[22] or 'stir bars' or molecular logic[23] to effect environmentally triggered release and control of the release rate profile.

The highly tunable nature of MSNPs has also provided an ideal platform for the development of even more advanced nanocarriers with specific and controlled release of their cargo.

Biocompatibility and Toxicity

A critical issue for any potential nanocarrier for medical applications is toxicity. The toxicity of silicon dioxide, both crystalline and amorphous, has been studied for more than a century, especially as it relates to *silicosis*, and recently, the toxicity of silica nanoparticles has been extensively investigated, due in part to the high surface-to-volume ratio of nanoparticles that could potentially lead to enhanced cellular interactions and different pathways of toxicity compared with coarse grained silica[15]. There is a general consensus that toxicity of MSNPs and amorphous silica in general is associated in part with the surface silanol groups, which can hydrogen bond to cellular membrane components or, when dissociated to form SiO^- (above the isoelectric point of silica ~pH 2-3), interact electrostatically with the positively charged tetraalkylammonium-containing phospholipids, both processes leading to strong interactions and possibly membranolysis[24].

Based on the high surface-to-volume ratio of silica NPs, it might be anticipated that they would show in general higher toxicity compared with their bulk counterparts (e.g., crystalline or amorphous). However in the case of MSNPs, the intrinsic porosity of the MSNP surface reduces the extent of hydrogen bonding or electrostatic interactions with cell membranes[24]. Considering both former and latter facts about silica in a nanoparticulate form, it would seem unclear as to the potential toxicity that MSNPs would display. With this in mind, many studies have been performed recently to address this.

Although the porosity of MSNPs should decrease their toxicity due to the decreased surface interaction, studies of the toxicity of MSNPs have shown widely variable ranges of toxicity. One potential reason for the variability in toxicity studies is the surfactant used to template the pores is toxic and variable amounts of this surfactant can remain within the pores of the MSNP depending on the processing[25]. A recent study which used FTIR to confirm that the template surfactant had been removed prior to testing MSNPs for toxicity found survival of all mice treated with up to 1000mg/kg by IV injection and followed for 14 days[26]. The survival of all the animals treated with a very high dose of MSNPs that did not retain surfactant shows the lack of toxicity of the silica framework of the MSNP itself. Potential toxicity is further mitigated by the high drug loading capacity of MSNPs, which greatly reduces needed dosages compared with other nanocarriers. Studies of drug loaded MSNPs in mice have shown that they are well tolerated and demonstrated no histological changes in organs at therapeutic doses such as 1mg/kg IV injection[26]. Mice treated with MSNPs with or without a PEG coating at higher doses, such as 20mg/kg IV injection, also demonstrated no signs of toxicity and no organ damage visible by histology[27]. Additionally, the ability to modify the surface of MSNPs with polymers or lipids will alter and potentially reduce toxicity of MSNPs. Finally, the ability to add targeting will further modify and reduce toxicity as the MSNPs are directed specifically to the target cells or tissues of interest and will have reduced nonspecific interactions within the body as a whole. Regardless, it is important to test all proposed nanocarriers in their final form for toxicity as well as to take into account the highly tunable and variable options presented by the MSNP platform. In addition to toxicity, the biocompatibility of the nanocarrier must also be taken into account. In this area, the porous structure of the MSNPs further enhances their biocompatibility as the high surface area and low extent of condensation of the MSNP siloxane framework promote a high rate of dissolution into soluble silicic acid species, which are found to be nontoxic[25]. The breakdown of the MSNPs overtime into nontoxic species supports the potential of repeat and long term use of the MSNPs to deliver drugs as the MSNP can be cleared from a biological system, overtime, in a nontoxic way. Examination of animals treated with both PEG coated and unmodified MSNPs showed excretion of the silica in both feces and urine[27]. The safety of MSNPs is also supported by the fact that amorphous silica is Generally Recognized as Safe (GRAS) by the FDA. Recently amorphous silica nanoparticle 'C-dots' (*Cornell Dots*) were FDA approved for diagnostic applications in a stage I human clinical trial[28]. The FDA clearance for a clinical trial of silica nanoparticles should accelerate the acceptance of amorphous colloidally derived silica's for applications in medicine.

In Vivo Application of Mesoporous Silica Nanoparticles to Cancer Models

The study of MSNP as nanocarriers has advanced in recent years to studying the capacity of MSNPs to successfully deliver cargos to *in vivo* animal models of human cancers. Some of current studies have focused on the use of the enhanced permeability and retention (EPR) effect found in tumors. Meng *et al.* showed that the addition of PEG to the surface of MSNPs loaded with doxorubicin allowed 12% of the particles to accumulate within a tumor xenograft. In this study, the treatment response, of mice bearing squamous cell carcinoma xenografts, to the PEG coated doxorubicin MSNPs were compared to free doxorubicin, which showed an increased efficacy of the MSNPs versus the free drug. The mice in the study also showed reduced side effects, including reduction in weight loss as well as reduced liver and renal injury from the drug loaded MSNPs versus the free doxorubicin treatment[15]. More recent studies have begun to take advantage of the ability to add targeting moieties to the surface of the MSNPs. He *et al.* targeted polymer coated MSNPs to cervical cancer cells by conjugating transferrin to the MSNPs and increased the uptake of the MSNPs by also conjugating TAT cell penetrating peptide to the surface of the MSNPs. These targeted MSNPs were able to successfully deliver selenocysteine as a synergistic chemo- and radiotherapy agent to cervical cancer xenografts. Selenocysteine is a potential anticancer agent whose clinical development has been hindered by low selectivity, solubility and stability issues, which potentially could be overcome by loading the selenocystine into MSNPs. Mice treated with the targeted selenocystine MSNPs had dose dependant decreases in tumor volume at lower doses than mice treated with free selenocystine, showing the increased efficacy of the targeted MSNPs versus free drug[26]. The use of MSNPs has even been explored for increasing vascular access in difficult cancer types such as pancreatic ductal adenocarcinoma (PDAC). PDAC elicits a dense stromal response that limits the vascular access to the tumor and contributes to chemotherapy resistance. Polyethyleneimine (PEI)/polyethylene glycol (PEG) coated MSNPs containing the TGF-β inhibitor, LY364947, were delivered first to decrease pericyte coverage of the vasculature. The MSNPs were then followed by treatment with liposomes containing gemcitabine, a first line chemotherapy agent. The high loading capacity and pH-dependent LY364947 release from the MSNPs facilitated rapid entry of IV-injected gemcitabine containing liposomes and MSNPs at the PDAC tumor site. This two-wave approach provided effective shrinkage of the tumor xenografts compared to the treatment with free drug or gemcitabine-loaded liposomes only[29]. As shown by these studies, the utility and the variety of MSNPs for increasing drug delivery and specificity is increasing rapidly. As such, MSNPs have promise for decreasing toxicity for many chemotherapy agents and potential for increased efficacy in difficult to treat cancers.

Future Developments

The modular design of mesoporous silica constructs promises a new drug and disease agnostic platform technology for customized delivery and controlled release of multiple types of cargos and cargo combinations. Packaging within MSNP will enable the re-purposing of drugs that have to date failed clinical trials due to poor solubility, high toxicity, and/or susceptibility to degradation. MSNP supported lipid bilayers (so-called *protocells*) have the further advantage that the bilayer can retain and protect fragile and/or highly soluble cargos and enable triggered release of the cargo upon acidification within the tumor or tumor microenvironment. The modularity of the MSNP size, shape, pore size and surface chemistry further suggest applications in personalized medicine requiring individualized cargo combinations, targeting, and release profiles. However the modularity and versatility of MSNP may pose difficulties in pursuing FDA approval as new standardized protocols will be needed to establish structure, cargo content, PK/PD, and degradation profiles.

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Milestones to address these critical areas that researchers should be able to be achieve over the next 5-15 year time frame include many aspects. In the next 5 years, researchers will establish standardized procedures to characterize the physicochemical properties of MSNPs including purity, cargo loading and release, and biodegradation; Determine the size, shape, and surface chemistry dependence of the biodistribution, biodegradation and toxicity (e.g. maximum tolerated dose) of non-targeted MSNP depending on the route of administration and cancer model in small animals and dogs; Demonstrate the *in vivo* performance of targeted MSNP for delivery of multiple types of cargo to tumors and circulating and metastatic cancers in small animals; Perform PK/PD studies of select MSNP and targeted MSNP in small animals to correlate therapeutic efficacy with MSNP nanostructure and cargo loading and release characteristics; and conduct Phase 0 clinical trials of select non-targeted MSNP for delivery of small molecule cargos such as doxorubicin, paclitaxel, or cisplatin and cargo combinations. Looking further ahead over the next 10 years, researchers will conduct phase 0, I, and II clinical trials for select MSNP/cargo combinations and optimize MSNP performance (BD and PK/PD) via re-engineering of physicochemical properties; gain FDA approval of at least one MSNP-based therapeutic; and conduct phase 0, I, and II clinical trials for targeted MSNPs and MSNP theranostics and optimize *in vivo* performance. Looking further ahead over the next 15 years, researchers could gain FDA approval of at least twenty MSNP-based therapeutic systems including targeted MSNP, combination cargos, and theranostics; and conduct phase 0, I, and II clinical trials for personalized MSNPs with individualized cargos and targeting.

In Vivo Self-Assembly/Disassembly of Nanoparticles for Cancer Imaging and Drug Delivery

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Introduction

Nanoparticles have been shown to offer great detection sensitivity because of their unique physical, optical, electrical, and magnetic properties. Enormous efforts have been made in designing and synthesizing a variety of nanoparticles and applying them to cancer imaging. However, translation of nanoparticles-based contrast agents to clinical cancer imaging has been challenging, as summarized in a recent opinion paper authored by the NCI Alliance for Nanotechnology in Cancer Imaging working group[30]. Intravenous infusion is the most common delivery strategy for anticancer therapy or imaging applications. Injected nanoparticles have often met hurdles, such as non-specific uptake by the reticuloendothelial system (RES) and long-term retention in the body leading to chronic toxicity. The tools available to mitigate these effects are limited. A commonly used approach to reducing RES uptake and increasing circulation times is steric stabilization of particle dispersions by polyethylene glycol (PEG) coating. However, long circulation times achieved by PEG-coated "stealth" particles do not necessarily lead to enhanced accumulation deep into tumors because the relatively large size of nanoparticles attenuates transvascular transport and interstitial penetration (**Figure 3** left). To overcome these challenges, nanoparticle design and delivery have to be optimized, which is the main focus of the nanoimaging field. We have been exploring a unique approach to developing novel nanotechnology that will have high translational potential to clinical cancer imaging.

Our new, unique approach explores the concept of directly building nanoparticles inside living cells from small molecular weight building blocks taken up by target cells, as outlined in **Figure 3** (right). Small molecules typically have good transvascular transport and interstitial penetration into tumor (**Figure 3** middle), but unfortunately they are poorly retained at the target site and easily washed out. This new strategy seeks to combine the advantages of nanoparticles and small molecules for cancer imaging and drug delivery. More specifically, small molecules are injected through intravenous infusion, so they will diffuse into the interstitial space after crossing through the vascular vessels in the tumor. To enhance their retention in the tumor, they are activated by tumor-specific biomarkers already present and self-assemble into nanoparticles. At other tissue locations, where the cancer-specific biomarkers are absent, activation and the subsequent self-assembly does

not occur. Thus, the injected small molecules are poorly retained relative to the assembled nanoparticles at the tumor site. This new nanotechnology will help provide solutions to many challenges encountered in nanotechnology based drug delivery and cancer imaging.

Current State in the In Vivo Self-Assembly of Nanoparticles

This concept was first demonstrated in fluorescence imaging of the activity of a furin-like convertase in cell culture[31]. The success was enabled by a novel bioorthogonal reaction between an aromatic cyano group and a 1,2-aminothiol group[32]. The amino and thiol groups are conjugated with a masking group, and only after activation by the target enzyme to generate the free cysteine, will condensation take place to form macrocycles. These macrocycles have very affinity for each other and not the surrounding medium, thus readily self-assemble into nanoparticles. The end result being extended signal enhancement and retention in the local region where they assembled. Two modes have been established in the molecular cascade which enable this nanoparticle self-assembly: intermolecular condensation[31,33,34] and intramolecular cyclization[35–39]. Both initial condensations are specific, and with the subsequent intramolecular cyclization, it is free from any potential competition by endogenous free cysteine[35].

Since then, it has been shown that this approach can be applied to image many molecular targets and is compatible with a range of imaging modalities such as fluorescence[37], photoacoustic[34], magnetic resonance imaging (MRI)[33,38,39], and positron emission tomography (PET)[36]. For example, we have successfully synthesized a [^{18}F]-labeled caspase-sensitive nanoaggregation PET tracer ([^{18}F]-C-SNAT), and have validated it for PET imaging of caspase-3 activity with a doxorubicin-induced tumor apoptosis model in nude mice bearing HeLa tumor xenografts[36]. Using a super-resolution fluorophore, we have directly visualized the assembled fluorescent nanoparticles in apoptotic tumors, and thus fully validated the working mechanism *in vivo*[37]. We have shown that different biomolecules such as caspase-3/7[36–38], furin[32,34,35], beta-galactosidase [unpublished], and redox changes[33,39] can specifically remove the masking groups to trigger the condensation reaction and self-assembly.

These studies have clearly demonstrated that this *in vivo* target biomolecule-triggered self-assembly platform could be transformative for clinical cancer imaging. Because the nanoparticles are generated *in situ* at the cancer target site, the small molecule precursors will not encounter the same challenges faced with current injected nanoparticle-based *in vivo* diagnostic contrast agents. Rather, these nanoparticles are selectively synthesized at the tumor site to enhance imaging contrast.

Notably, a group at Brandeis University has developed a different chemical system, albeit based on the same concept, to generate pericellular and intracellular nanofibers for antitumor activity. The monomers used in this system are small peptides that are highly water-soluble. These small peptides are the substrate of a target enzyme such as alkaline phosphatase found in the cell. Upon the enzymatic processing of the small peptides, they will self-assemble into nanofibers through hydrophobic interactions at a site that is near the enzyme. With respect to their potential efficacy, it has been reported that the formation of nanofibers can lead to death of cancer cells *in vitro* through disruption of the dynamics of microtubules[40].

Another group at the University of Toronto has explored this *in vivo* nanoparticle assembly concept through a biotin-streptavidin interaction[41]. In their studies, poly(ethylene glycol) (PEG)-grafted small nanoparticles bearing biotin and streptavidin-conjugated fluorescent probes are injected sequentially. Both are diffusive and permeable to the tumor vasculature, and upon co-localization, they assemble into nanoaggregates, which is mediated via the strong biotin-streptavidin interaction, and enhance retention at the tumor site.

Future Scientific and Clinical Developments

Our current research has established an *in vivo* self-assembly nanoplatform for cancer diagnostics. To further advance this novel platform, one very critical component would be to introduce a novel design element that would allow for a gradual *disassembly* of the assembled nanoparticles into small molecules again, at the end of imaging. The purpose of this would be to allow

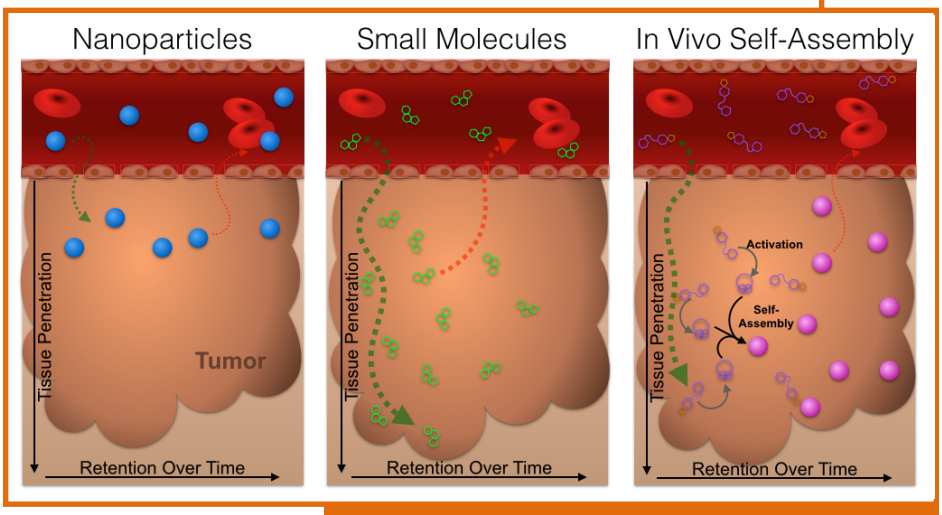

Figure 3. Schematic of transvascular transport and interstitial penetration of three types of intravenously injected materials. Left: nanoparticles cross the leaky tumor vasculature and are trapped well, but poorly penetrate due to its large size. Middle: small molecules (e.g., drugs) diffuse and penetrate deeply, but are poorly retained. Right: a new type of small molecules can be activated to self-assemble into nanoparticles after diffusion and penetration into tumor.

the nanoparticles to be eliminated from the body post-imaging. As such, over the next 5 years, this will be a primary focal point in this field, i.e., to establish *in vivo* disassembling technology and integrate it into the current self-assembling platform for cancer imaging in pre-clinical animal models. This self-assembly/disassembly nanoplatform will be applied to a range of cancer-specific targets and produce a number of imaging probes successfully evaluated in small animals.

In the next 10 years, those most promising Phase 0 candidates should be able to be further translated into human applications in the clinic as they will reach IND stage for clinical testing. It is expected that the unique feature—*in vivo* self-*assembly*/*disassembly* of nanoparticle—of these nanoplatforms should overcome the challenges commonly associated with injected nanoparticles, such as the transendothelial barrier to delivery, and minimize the acute and chronic toxicity, which is the primary reason for an optimistic view of their facile translation to the clinic.

...the small-molecule nature of these agents should present an important advantage for commercialization and large-scale production.

In the next 15 years, some of these agents will gain FDA approval for clinical applications such as cancer diagnosis, patient stratification, treatment monitoring and imaging-guided surgery. Moreover, the small-molecule nature of these agents should present an important advantage for commercialization and large-scale production.

DNA/RNA-Based Nanostructures for Cancer Nanomedicine

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Nucleic Acid Nanotechnology

Over the past several decades, nucleic acid molecules (DNA, RNA and their chemical cousins and derivatives) have emerged as highly programmable building blocks for nano-construction due to the increasing knowledge of their three-dimensional (3D) conformations and intra- and inter-molecular base pairing interactions[42]. A variety of design rules and assembly methods have been developed to engineer self-assembling nucleic acid nanostructures of increasing complexity[43,44]. DNA nanostructures ranging from periodical lattices to discrete objects of various sizes have been constructed using a rich library of DNA nanostructure motifs and different assembly strategies[43]. DNA origami, a method that uses a number of short, single-stranded DNA (ssDNA) oligonucleotides to direct the folding path of a long ssDNA 'scaffold' strand, has enabled the construction of spatially addressable and geometrically sophisticated 2D and 3D DNA nanostructures with near-quantitative yield[45–47]. As the sister molecule to DNA, RNA has also shown great promise in engineering rationally designed nanostructures. The canonical and non-canonical base pairing interactions, as well as the greater diversity of tertiary structures resulting from a rich library of naturally existing RNA structural motifs, have led to an emerging field of RNA nanotechnology[44,48,49]. Nucleic acid analogs such as PNA (peptide nucleic acid), LNA (locked nucleic acid), GNA (glycol nucleic acid) and TNA (threose nucleic acid), and chemical modifications of nucleic acids have all brought useful properties, including improved chemical, biological and thermo-stability to nucleic acid nanostructures. The structural properties of nucleic acid, which allow it to serve as a versatile construction material, have also been exploited to create dynamic nanodevices ranging from small switchable structures to structures that display complex motions[50]. In addition, logic gates and molecular computing based on nucleic acid building blocks have opened up great opportunities to implement sense-compute-actuate mechanisms into nucleic acid based nanosystems[51]. This is highly desirable for developing intelligent molecular devices for biological and medical research.

Nucleic Acid Nanostructures for Cancer Nanomedicine

The ability to engineer designer DNA nanostructures with high programmability and accurate spatial and dynamic control has allowed researchers to explore novel applications

in cancer nanomedicine. Nucleic acid nanostructures are attractive materials for this purpose, not only because of their inherent design modularity, structural programmability and biocompatibility, but also because nucleic acid molecules of a particular sequence can be modified to selectively bind, distinguish and communicate with target cells to trigger controlled delivery of therapeutic agents. With the development of various chemical conjugation methods, it is now technically feasible and convenient to present functional molecules, such as proteins or peptides, nucleic acids (aptamers, anti-sense RNA, siRNA etc.), inorganic nanoparticles (metallic, semiconducting and magnetic nanoparticles) and organic fluorophores at selected sites on nucleic acid nanostructures for making programmed theranostic devices. For example, researchers recently developed a DNA nano-barrel with single stranded aptamer locks that were opened to expose the loaded antibody cargo only in the presence of target cells[52]. Performing molecular computation directly on the surface of cells, or in cellular environments, will facilitate *in vivo* targeting and drug release. Recently, Rudchenko, Stojanovic and colleagues engineered DNA strand displacement cascades that detected the presence of certain biomarkers on the surface of cells[53]. In another report, Hemphill and Deiters successfully engineered oligonucleotide logic gates to detect specific microRNA inputs in live, mammalian cells[54]. As more complex and robust nucleic acid based computing systems are developed, it may be possible to integrate them into cellular systems to control and trigger cellular functions, such as gene expression, or to interfere with the metabolic pathways. By combining nucleic acid computation-based target cell detection with reconfigurable nucleic acid nanostructure-based drug containers, it may be possible to create a nucleic acid-based nanorobot that can interface and communicate with living cells to develop smart cancer therapy.

A critical step in administering effective drug therapy is the initial delivery of the therapeutic agents into cells. It was found that some nucleic acid nanostructures can be directly and efficiently internalized into live cells without transfection agents[55]. Although the underlying mechanisms still remain to be explored, such cell-penetrating nucleic acid nanostructures, in combination with targeted ligand-receptor recognitions, may lead to the development of universal cellular delivery systems. Pure DNA nanostructures have already displayed higher structural stability and resistance to nuclease digestion[56,57], compared to double helical DNA molecules. Recent studies further demonstrated that enclosing DNA nanostructures with PEGylated lipid bilayers leads to enhanced protection against nuclease digestion with decreased immune activation and significantly improved pharmacokinetic bioavailability[58].

There are several studies that have utilized the unique structural and geometric features of DNA nanostructures to deliver DNA or RNA molecules into cells (**Figure 4**). Examples include the delivery of DNA nanostructure-scaffolded CpG oligonucleotides *in vivo* to trigger immune responses[59] and delivery of siRNA both *in cellulo* and *in vivo* for regulation

of protein expressions[60]. DNA nanostructures carrying chemical drugs such as Doxorubicin have demonstrated great value in not only efficient drug delivery, but also simultaneously circumventing the drug resistance problem in chemical therapy[61].

Several unique properties, such as higher thermostability and synthesis scalability through *in vitro* and *in vivo* transcription, have made RNA-based nanostructures appealing molecular scaffolds for cancer therapy applications. In addition, the chemical stability of RNA nanostructures has been greatly enhanced by introducing chemical modifications such as the 2'-Fluro substitution to the 2'-OH group. It has been shown that a RNA-based nano-scaffold displays favorable pharmacokinetic profiles *in vivo* and shows no toxicity in mice[62]. Exemplified by the utility of the phi29 pRNA nanostructure system, RNA nanoparticles carrying various ligands such as siRNA, micro-RNA, and aptamers have shown great promise in targeted delivery of cancer therapeutics[63]. More recently, a multi-module

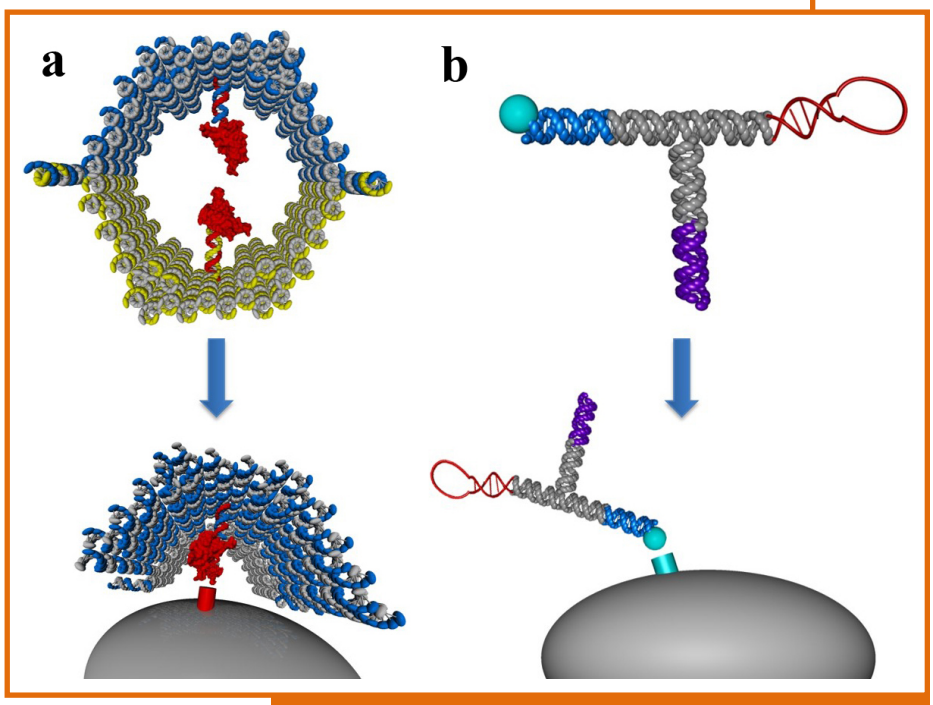

Figure 4. Programmable multi-functional nucleic acid nanostructures for cancer therapeutics. (a) Schematics illustrating the use of a DNA nanocage for targeted recognition of cancer cells. Top: Closed DNA nanocage loaded with an antibody payload. The cage is set to the closed state using structural switching DNA aptamer locks. The aptamers recognize the receptor molecules on the cancer cell surface to trigger the unlocking of the cage to expose the antibody to the target cell. Other payloads, such as chemical drugs, siRNA, and micro-RNA may also be loaded to create multi-functional targeted cancer therapeutics. (b) Illustration of a multi-functional three-way RNA junction motif carrying folate for cancer cell recognition, malachite green dye binding aptamer for cell imaging and siRNA for cancer cell gene expression regulation.

...nucleic acid based nanostructures can also be explored for cancer immunotherapy, ranging from immune activators, tumor-specific vaccines to immunosuppression blockers.

pRNA nanoparticle functionalized with folate acid was constructed to actively target metastatic cancer cells, demonstrating its benefits in treating cancer metastasis[64].

Given the intrinsic adjuvant activity of DNA and RNA molecules, nucleic acid based nanostructures can also be explored for cancer immunotherapy, ranging from immune activators, tumor-specific vaccines to immunosuppression blockers. Initial research in this direction includes the assembly of model vaccines using nucleic acid nanoscaffolds that display multiple immunogenic molecules and deliver immune-stimulating molecules to cells[59]. Yan, Yung and co-workers have demonstrated good immunogenicity of DNA-scaffolded vaccines. With a growing number of immune activators and check-point blockers being identified, one can use nucleic acid based-nanostructures to rationally assemble these molecules for elicitation of stronger and more effective anti-tumor immunity. Thus, the application of nucleic acid based nanostructure platforms for directed assembly of synthetic vaccines and immune modulators has great potential to revolutionize cancer immunotherapy. Furthermore, many chemotherapeutic drugs have been shown to enhance anti-tumor immunity, *via* an induction of immunogenicity of cell death and selective killing of immunosuppressive cells. Thus, programmable nucleic acid based nanostructures are best suited for the development of combined chemo- and immunotherapeutics in our fight against cancer.

Future Developments

To realize the full capability of using nucleic acid nanostructures for cancer research and treatment, several critical issues need to be addressed and carefully investigated. First, although initial studies have shown that some nucleic acid nanostructures (modified or unmodified) do not trigger strong immune responses, the safety of a larger spectrum of nucleic acid nanostructures must be established before practical use in clinical trials, given the adjuvant nature of DNA and RNA. Second, the use of nucleic acid based nanostructures for diagnostic and therapeutic applications rely on the complete clearance or degradation of the nucleic acid nanostructures within a reasonable amount of time. Depending on the type of application, it is important to investigate the bio-distribution, pharmaco-kinetic and dynamic (PK/PD) profiles of the nucleic acid nanostructures so that the nanostructures can be improved to achieve an optimal balance between efficient delivery and sufficient

retention time *in vivo*. Third, a set of design rules and parameters needs to be generalized for the nucleic acid nanostructure geometry, dimension, dynamics of reconfigurability, functionalization and chemical modification to develop the most effective nanodevices for different purposes of cancer therapy (e.g. structures need to be tuned to achieve balanced drug loading capacity and efficient targeted delivery; positions of recognition ligands on the nanoscaffolds need to be optimized to achieve improved affinity with minimized non-specific binding etc.). Fourth, a central obstacle to transforming nucleic acid nanostructures into clinical solutions is the cost of synthetic oligonucleotides. Researchers have made significant progress in producing RNA nanostructures through *in vitro* and *in vivo* transcription[65,66], and replicating small DNA nanostructures *in vivo*[67]. Further efforts are required to develop robust protocols to scale up the production of nucleic acid nanostructures of various designs through transcription, replication or through reducing the cost of nucleic acid oligo synthesis.

Indeed, a great advantage of using nucleic acid nanostructures for cancer nanomedicine is the ability to create multi-functional dynamic nanodevices with high programmability and intrinsic sequence/spatial addressability. There is plenty of room to take full utility of such a unique advantage for cancer nanomedicine. For example, nucleic acid nanostructures hold great potential to design and construct a set of novel, multifunctional, programmable anti-cancer vaccines that are specifically targeted to the tumor and programmed to release anti-cancer therapeutics and immune modulating factors at the tumor site to induce a robust, systemic immune response that will cause a sustained tumor regression. When such designs are integrated with molecular computing and programming, smart molecular doctors and personalized cancer therapeutics are within reach in the foreseeable future. Upcoming breakthroughs would require a multi-disciplinary effort from chemistry, biology, materials sciences, computer science, physics and clinical studies to push the boundaries of this exciting research area.

There is plenty of room to take full utility of such a unique advantage for cancer nanomedicine.

Milestones to address these critical areas that researchers should be able to be achieve over the next 3-10 year time frame include many aspects. In the next 3 years, researchers will evaluate the *in vivo* stability, bio-distribution and pharmaco-kinetics for a wide spectrum of nucleic acid nanostructures; identify optimal nucleic acid nanostructures with predictable behaviors *in vivo*; and develop robust and standard protocols to functionalize nucleic acid nanostructures to display therapeutic functions and targeted *in vivo* delivery properties. Looking further ahead over the next 5 years, researchers will evaluate the safety issue of the nucleic acid nanostructures which have demonstrated optimal *in vivo* behaviors;

develop multifunctional nucleic nanostructures and validate their initial uses in targeted cancer therapy and cancer vaccine development; and develop methods to scale down the cost of nucleic acid nanostructures and standardize protocols to make high yield synthesis of homogenous nucleic acid nanoparticles with designed functionality. Over the course of the next 10 years, researchers will conduct clinical trials of a variety of nucleic acid nanostructure-based cancer therapeutics; and integrate nucleic acid nanostructure-based therapeutics with molecular computing and programming to develop smart therapeutics in response to the cellular and tissue environments of various cancer and cancer matastasis.

Cooperative Nanosystems

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More Than the Sum of Its Parts

Bioengineers are currently designing increasingly sophisticated nanoparticles that can deliver treatments and diagnostics selectively to tumors[68,69]. Much of the field's focus has been on engineering the functionalities of individual nanoparticles to improve their transport[70], to target them to the tumor vasculature[71,72] or extracellular matrix[73], to deliver therapeutics[74,75], diagnostics[76], or heat[77,78] to the tumor environment, and to reprogram cancer cells[79] or the immune system[80]. However, the behavior of each nanoparticle depends not only on its design (size, shape, charge, material, cargo, and coating), but also on the interactions that occur in the body as a result of these design components. Thus, it is the collective, or 'systems' behavior of trillions of such nanoparticles interacting in a complex tumor environment that will define their success as diagnostic or treatment agents[81].

Predicting and engineering these collective nanoparticle behaviors is empirical and not always intuitive. For example, nanoparticles that are optimized to strongly bind and accumulate in cancer cells may mostly build up in the most proximal cells they encounter after leaking into the tumor environment. The resulting collective behavior is poor tissue penetration, leaving deep seeded tumor cells untreated[82–84]. Weaker nanoparticle binding, although detrimental to the function of the individual nanoparticle, could still lead to a better outcome by the system as a whole. Further engineering these behaviors on the level of single nanoparticles could result in emergent cooperative behaviors typically seen in self-organized systems[85].

Self-organized systems in nature, including those formed by social insects, animals, and cells, are able to perform complex behaviors through the local interactions of many simple agents and their environment[86–89]. The field of swarm robotics[90,91] has long taken inspiration from nature to engineer minimal robots that use simple rules to interact with their neighbors and local environment to solve complex real world problems[92–95]. Cooperative behaviors relevant to nanomedicine applications include amplification, optimization, mapping,

structure assembly, collective motion, synchronization and decision-making. By tapping into the field of swarm engineering, we may be able to produce behaviors that go beyond the functionalities of the individual nanoparticles and towards efficient, modular, and predictable system-based outcomes.

State-of-the-Art in Cooperative Nanosystems

Nanoparticles can cooperate implicitly, directly through self-assembly and disassembly, or through stigmergy (**Figure 5**). These behaviors have been useful to improve nanoparticle transport, accumulation, and distribution in tumor tissues towards development of treatment and diagnostic applications.

Most nanoparticle systems implicitly cooperate, in which each nanoparticle is designed to optimize its individual functionality[96]. The collective impact of the nanoparticles as treatment or imaging agents is assumed to be the sum of the independent nanoparticle effects. Understanding the system level behavior of implicit cooperators may add insight that can improve outcome predictions. Emphasis could be placed on studying whether the nanoparticles can collectively distribute throughout a tumor environment or accumulate at effective levels in, or around, targeted cells[70]. Similarly, combination therapies aimed at preventing resistance can be composed of different types of nanoparticles that independently target varied signal pathways, or even subpopulations within the tumor[97–99].

In addition to implicit cooperation, nanoparticles that physically interact harbor a more direct means of cooperation. Nanoparticles in this class of particles typically self-assemble or disassemble to modify their kinetics, or to collectively transport combined treatment and imaging agents to tumors. For example, rapidly diffusing imaging agents are able to anchor in tumors by binding to previously injected gold nanoparticles that have been given time to accumulate outside the vasculature via the EPR effect[40]. Similarly, small (10 nm) gold nanoparticles engineered to release conjugated doxorubicin in acidic tumor environments can subsequently self-assemble to form larger gold aggregates that are then available for use in photothermal therapy[100,101]. *In vitro* experiments reveal that nanoparticles capable of self-assembly in response to enzymatic activity may be able to perform logic computations towards the diagnosis of tumor state[102]. In another example, larger nanoparticles (100 nm) are able to disassemble into smaller nanoparticles once inside the tumor environment in response to enzymatic activity, thereby improving their circulation time, accumulation in the tumor, and ability to penetrate deep in the tissue[103]. Other multi-stage nanoparticles such as nested nanoparticles, mother ships, and nanocells are all able to overcome transport barriers through the release of nano-based components in tumor environments[104–106].

In contrast to collective behaviors mediated by direct interactions between nanoparticles, many swarm systems found in nature communicate by modifying the environment. This concept is called stigmergy[86]. Ants deposit and sense chemical signals to form trails that lead to sources of food[87]. Termites are able to build complex structures by modifying and locally sensing their physical environment[94]. In a similar way, nanoparticles have been designed to modify their physical environment or deposit signals. Gold nanorods that accumulate in a tumor, upon heating to sub-lethal temperatures with NIR light, can improve perfusion of angiogenic vessels and in some cases upregulate receptors used in targeting, which in turn improves the delivery of a second wave of nanoparticles, such as liposomes and magnetic nanoworms, to tumors for treatment and imaging purposes[107,108]. Gold nanorods heated through NIR light can also cause a clotting cascade in tumors[109]. This biological cascade serves as a signal to communicate the location of the tumor to circulating nanoparticles, thereby leading to a 40-fold increase in the amount of chemotherapeutic delivered to the tumor when compared to a non-communicating system[109]. Nanoparticles that aim to normalize the vascular bed, or degrade the extracellular matrix can improve the transport of secondary nanoparticles[110,111]. Nanoparticles can also be designed to release either a cargo or energy, which can directly interact with neighboring nanoparticles. As an example, gold nanorods activated through NIR light emit heat in tumors to trigger the release of chemotherapeutics contained in thermally sensitive drug carriers[112].

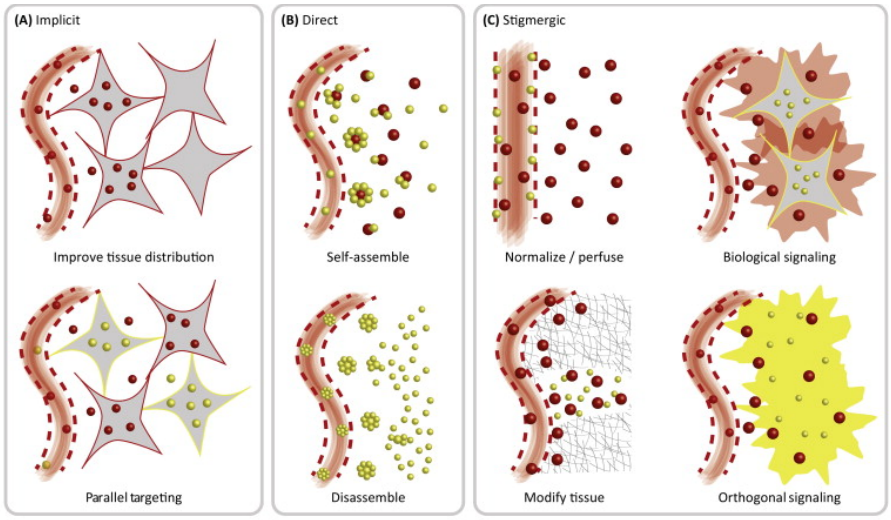

Figure 5. Mechanisms of cooperation in cancer nanomedicine. Nanoparticles can cooperate implicitly to improve their tissue distribution, directly through self-assembly and disassembly to change their distribution, or by communicating through the environment (stigmergy). Using stigmergic interactions, nanoparticles can impact perfusion or tissue density to improve the delivery of secondary nanoparticles. They may also communicate by initiating a biological cascade that can be sensed by other nanoparticles, or send an orthoganal signal (energy, chemicals) to activate secondary nanoparticles. (*Images and text reused with permission, Hauert and Bhatia, 2014*).

Systems Nanotechnology

The practice of engineering and predicting the collective behavior of large numbers of nanoparticles that interact in complex tumor environments is typically non-intuitive, even for simple nanoparticle designs. By harnessing a systems approach, bioengineers could start by automatically exploring potential nanoparticle designs using crowdsourcing (http://nanodoc.org) and machine learning[113], then modeling the resulting collective behavior in simulation[70,82,83,114], followed by testing the best candidates experimentally through fast prototyping of both the nanoparticles[115,116] and their environment[117], and finally validating the collective behaviors *in vivo* with feedback on their outcome provided by high resolution imaging[118]. Through this systems-based process (**Figure 6**), we expect nanoparticles to become more robust in their ability to react to environmental feedback by changing their motion and trajectory, thereby achieving increasingly swarm-like behaviors. Growing expertise in control of nanomaterials, achieving a deeper understanding of cancer biology, and ongoing advances in the modeling and automation of nanosystems are all contributing to the field's first steps in this direction.

More broadly, we anticipate that lessons learned from efforts made to design cooperative nanosystems will also prove useful in the engineering of naturally swarming biological components, such as cells of the immune system[119] or synthetic bacteria[120] in order to improve tumor treatment and diagnostics.

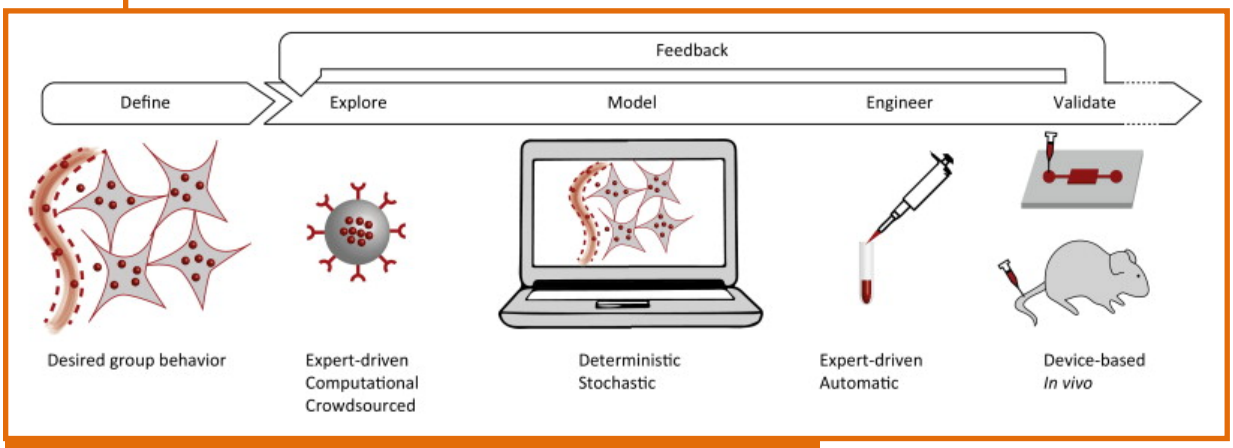

Figure 6. Systems approach to the design of cooperative nanomedicine. Starting from a desired group behavior, tools are needed to explore possible nanoparticle designs, model their resulting cooperative behaviors in simulation, engineer the nanoparticles, and validate them *in vitro*, and *in vivo*, before clinical translation. (*Images and text reused with permission, Hauert and Bhatia, 2014*).

Multimodal Imaging Constructs

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Introduction

With the aim in mind to create molecular imaging beacons that can be "seen" by multiple imaging methods, nanoparticles have several key advantages over small molecule contrast agents: (1) It is possible to integrate multiple contrast agents into the a single nanoparticle, and therefore combine their complementary strengths (e.g., whole body imaging and high resolution during intraoperative imaging). It is not possible, however, to simply mix the contrast agents together and expect reasonable signal to be generated for each modality. Most contrast agents require a particular environment to achieve optimal performance. Nanoparticles are small enough so they can be tuned to reach tissues of interest, but also large enough so that the particular needs of each contrast agent can be met within the same particle. (2) Their size range is ideal so that they can be coated with a variety of surface modifying moieties. These moieties can range from antibodies, affibodies, peptides or small molecules in order to induce binding of the particles to a specific target of interest. Here, the clustering of a large number of such targeting moieties on the relatively small surface of the nanoparticle can amplify their targeting abilities via multivalency effects. Nanoparticle surfaces can also be passivated with other moieties (e.g., polymers), through which one can influence and fine-tune the blood half-life and overall whole body biodistribution. (3) Nanoparticles can also be "armed" with many different therapeutic functions, be it that they deliver drugs at the target site or that they serve as photothermal agents that can destroy tumor cells via heat induction.

Current State for Multimodal Imaging Via Nanotechnology

There has been significant progress in the design and application of multimodal nanoparticles since 2010. One of the first nanoparticles that were in clinical trials for imaging purposes are superparamagnetic iron oxide nanoparticles (SPIONs)[121,122]. While several different versions with slightly different chemical compositions were in clinical trials for lymph node imaging with MRI these never received full FDA approval, and were subsequently taken off the market[121]. It is well known, however, that the iron contained in SPIONs is incorporated into the iron pool of the human body upon degradation of the particles, and the formulation as a nanoparticle can be more efficient than elemental iron in replacing iron in humans. This lead to the FDA approval in 2009 of a modified formulation

(Ferumoxytol) for the treatment of iron deficiency anemia in adult patients with chronic kidney disease. While not yet approved for imaging purposes, this has lead to a renaissance of clinical studies using SPIONs as an MRI contrast agent (e.g., NCT01336803). Given the many preclinical studies that used SPIONs as a platform for multimodal imaging, such as by adding a fluorochrome or radiotracer, this also rekindles the hope that such multimodal nanoparticles will eventually receive approval for diagnostic imaging purposes[123,124].

Several nanoparticle therapeutics made of other materials such as gold, silica or both, are currently in advanced stages of clinical trials[125]. These advances are not only representing milestones in the field of nanotherapeutics, but also increase the likelihood of nanoparticles of similar size and composition to be approved for imaging purposes. In fact, in 2010 the FDA approved an IND for the first in human testing of so-called 'Cornell dots' or C dots (NCT01266096). C dots are silica nanoparticles that are less than 8 nm in size, contain fluorochromes in their core, and can be functionalized with radiotracers for PET imaging for dual modality detection of melanoma metastases[28]. This was the first time that the FDA approved a clinical trial using an inorganic material in the same fashion as a drug in humans.

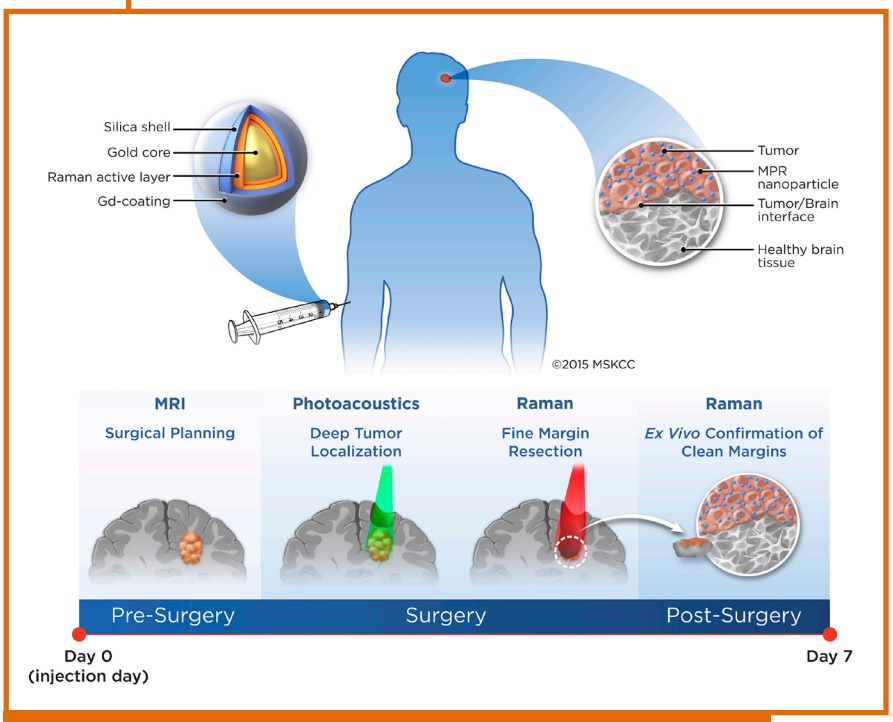

Figure 7. Principle of a triple-modality MRI-photoacoustic-Raman nanoparticle and its envisioned clinical use. The nanoparticle is injected intravenously. In contrast to small molecule contrast agents that wash out of the tumor quickly, the nanoparticles are stably internalized within the brain tumor cells, allowing the whole spectrum from preoperative MRI for surgical planning to intraoperative imaging to be performed with a single injection. T1-weighted MRI depicts the outline of the tumor due to the T1-shortening effect of the gadolinium. During the surgery, photoacoustic imaging with its greater depth penetration and 3D imaging capabilities can be used to guide the gross resection steps, while Raman imaging can guide the resection of the microscopic tumor at the resection margins. Raman could also be used for rapid confirmation of clean margins in the operating room instead of the time-consuming analysis of frozen sections.

Major advances have also been made in the

preclinical arena, of which only few can be mentioned in this short summary. These comprise improvements to existing modalities, integration of multiple modalities into the same nanoparticle, and the establishment of new imaging modalities. As an example of the latter, "surface-enhanced Raman scattering" (SERS) nanoparticles were shown for the first time to allow imaging of cancer and image-guided tumor resection[126]. It was also shown that such SERS nanoparticles could be transformed into multimodal molecular imaging agents, by adding detectability from both MRI and photoacoustic imaging. This triple-modality approach was developed, with the goal in mind, to perform more precise brain tumor imaging and image-guided resection (**Figure 7**). While the MRI capabilities allow for preoperative planning, intraoperative photoacoustic imaging can provide a surgeon with a roadmap for the gross resection steps, while SERS imaging indicates whether or not the tumor tissue has been completely resected at the microscopic level[126,127]. Because SERS provides such a specific signal (Raman "fingerprint"), it is ideally suited for high precision cancer imaging. This has more recently been demonstrated with a new generation of "surface-enhanced resonance Raman scattering" (SERRS) nanostars that are orders of magnitude brighter and allow imaging of microscopic disease in multiple different cancer types[128,129]. New synthetic protocols now allow the creation of multiple layers of silica, each fine-tuned in thickness and each containing a different contrast agent (patent pending). This principle allows incorporating a large number of contrast agents into the same nanoparticle, while also allowing optimal placement of each contrast agent within the particle architecture. For example, a SERS reporter has to be placed as close as possible to the noble metal core, while a fluorochrome has to be placed at a certain distance to avoid quenching of the fluorescence. An MRI contrast agent is ideally placed at the nanoparticle surface to allow interaction with water molecules. This principle is illustrated in **Figure 8**.

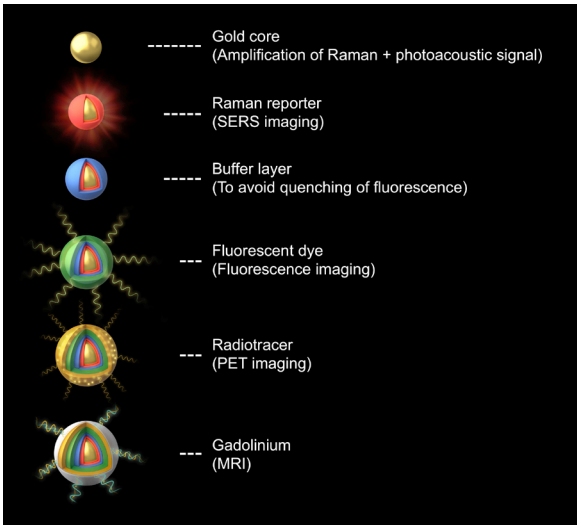

Figure 8. Synthesis of multimodal nanoparticles via a multilayer silication method. Addition of multiple layers of silica with finely tuned thickness as a strategy to incorporate many different imaging modalities into the same nanoparticle, while optimizing the signal intensity of each modality.

Future Challenges in Multimodal Imaging

The main challenge for nanoparticle imaging agents is and remains the regulatory approval by the FDA. Multimodal nanoparticles are facing significantly greater hurdles in the approval process than small molecule agents that would suffice for isolated PET, CT, MRI or fluorescence imaging. The most difficult hurdle for nanoparticles that are not small enough to be cleared via the kidneys is that sufficient proof has to be presented to the FDA that the retention of the nanoparticles in the body does not represent a health risk. Most intravenously injected nanoparticles are cleared from the blood by the organs of the reticuloendothelial system, such as the liver, spleen and lymph nodes, and are retained in these organs for extended amounts of time. In the case of SPIONs, Ferumoxytol has proven to be degraded over time, which facilitated regulatory approval. For those nanoparticle compositions that do not degrade or are eliminated from the body over time, it has to be shown that the retention does not cause any adverse effects. To this end, the recent development of novel artificial organoids that closely recapitulate human organs might offer a great avenue to accelerate such studies without having to risk the health of human patients.

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Milestones to address these critical areas that researchers should be able to be achieve over the next 3-10 year time frame include many aspects. In the next 3 years, researchers will conduct large animal studies of currently available multimodal imaging agents; initiate more clinical trials; and continue the development of next generation nanoparticle imaging agents. Looking further ahead over the next 5 years, researchers will test the newest generations of multimodal nanoparticles in artificial organs, which are expected to exist by then and should facilitate the translation into the clinics; and complete the currently ongoing clinical trials, analyze results and detail the lessons learned. In the next 10 years, multiple clinical trials should have been completed, including those that originated from initial testing in artificial organ systems. This should give a good indication about how well toxicity profiles can be predicted from studies in artificial organ systems, with the hope that parts of the current phases of the FDA required clinical trials can be replaced with testing in those novel model systems.

Theranostics: Smart, Multi-Functional Materials for Diagnosis and Therapy

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Overview

Current orthodox in the treatment of cancer involves surgical resection of large tumor areas followed by non-selective radiation therapy or chemotherapy. Such procedures can cause severe side effects from their non-specificity for tumor cells and concurrent damage to the immune system, rendering patients susceptible to other diseases. Moreover, the cancer frequently returns in refractory forms, resistant to current therapeutic approaches. Owing to the lack of effective late-stage cancer therapies, early detection and appropriate treatment is critical.

For the past two decades, the interesting and unique nanoscale delivery model and its respective tools have proven to be effective in medicine, especially in the field of cancer research and oncology. There has been much work to harness the tunable physicochemical properties of nanomaterials for diagnosis and therapy, such as real time visualization of cells/tissues and the precise delivery of therapeutic molecules to the targeted area. The diagnostic properties of nanomaterials (e.g., high plasmonic effect, enhanced MRI contrast effect, strong fluorescence, etc.) can enable early detection of small-sized tumors with exceptionally high sensitivity[130,131]. Furthermore, the multivalent characteristics of various nanomaterials allow for accurate tumor-specific imaging with the aid of a targeting moiety and synergistically integrated multi-modalities[132,133]. The improved targeting ability has also been advantageous from a therapeutic perspective, by which nanomaterials can selectively deliver therapeutic molecules to the tumor site, thereby increasing the therapeutic efficacy and reducing required dosages to minimize unwanted side-effects[71].

The distinct advantage of nanomaterials over conventional small molecules is their tunable physicochemical properties. Their size, shape, composition, and surface control can be adjusted to optimize their application in diagnosis and therapy. For example, rationally designed nanomaterials with specific dimensions and appropriate surface characteristics (e.g., neutral PEG and zwitterion) can circulate in blood vessels for a long time without opsonization by evading detection from macrophages and preferentially accumulate in tumor tissues via extravasation[134–136]. When incorporated with targeting moieties, the nanomaterials can be even more accurately delivered to the tumor site.

These phenomena are used for tumor-specific imaging (e.g., iron oxide for MR imaging and gold for highlighting tumor borders during brain surgery). As a method for enhancing diagnostic accuracy, multi-modal imaging (e.g., PET-CT and PET/SPECT-MRI) using different complementary modalities has been widely studied[133,137]. For example, nanoparticles functionalized with radioisotopes, known as multi-modal nanoparticles, have the potential to enhance diagnostic accuracy by increasing sensitivity of detection and adding the precision of anatomical localization[138]. Recently, magnetic particle imaging (MPI)-MRI demonstrates the potential for real-time visualization of tumor and cancer-related events (e.g., angiogenesis) with nano-molar sensitivity and anatomical details[139,140].

For therapy, the most promising and common application of these phenomena is the transportation of drug molecules. One example is BIND®, a targeted therapeutic nanoparticle, which in clinical trials has effectively reduced tumor sizes at lower doses than traditional chemotherapy[141]. The nanoparticles hold the chemodrugs without leakage during circulation and release them only upon reaching the targeted tumor. Some types of nanomaterials have additional therapeutic capabilities, such as the transformation of external energy to heat (e.g., iron oxide for magnetic fields and gold for light). These heat-generating therapies are known as photothermal ablation and magnetic hyperthermia, and they have been effectively used in cancer treatments[137,142]. The hyperthermia-based therapy has regulatory approval in 27 European countries[143].

Following treatment, nanomaterials can also be utilized to assess treatment efficacy and aid in making a prognosis (e.g., complete removal, regrowth, or metastasis of tumor). Nanosystems that can provide real-time diagnosis, in tandem with therapy and/or prognosis using multi-functional nanomaterials, are called *theranostics*. Research to combine the diagnostic and therapeutic characteristics of nanomaterials within a single platform, is being actively pursued. Currently, a wealth of research is being conducted in this area to improve cancer diagnosis and therapy. However, it is still only at the initial stages of the developmental pipeline.

Clinical Significance

From a diagnostic point of view, real-time monitoring of cancer-indicative markers (e.g., from genes and/or proteins) would allow for the administration of preemptive medicines at the moment pre-cancerous symptoms are found. A nanoparticle pill that Google is currently developing is a representative example of real-time monitoring[144]. When patients swallow a pill containing magnetic nanoparticles decorated with biomolecules for the identification of cancer or heart disease, the nanoparticle can detect and report signs of targeted disease through a wearable device. This proactive monitoring concept can switch the treatment

paradigm from the curative to the preventive. Even in cases where prevention fails, there is still a large benefit to early cancer detection. It keeps more effective treatment options available, which offers the best opportunity to be cured.

From a therapeutic point of view, the targeted delivery of therapeutic molecules to a tumor using nanomaterials can potentially enhance the efficacy of therapy and significantly reduce systemic toxicity, such as that experienced with Abraxane®, the FDA-approved paclitaxel albumin-stabilized nano-formulation[145]. When combined with the imaging capabilities of nanomaterials, the therapy can be monitored for maximum accumulation time, effective release of the drug, and the patient's response to treatment. This in turn allows for more informed decision-making on timing, quantity, type of drugs, type of drugs, and choice of treatment procedure, as well as an evaluation of an individual's response to treatment. This could be the basis for the future of personalized cancer treatment.

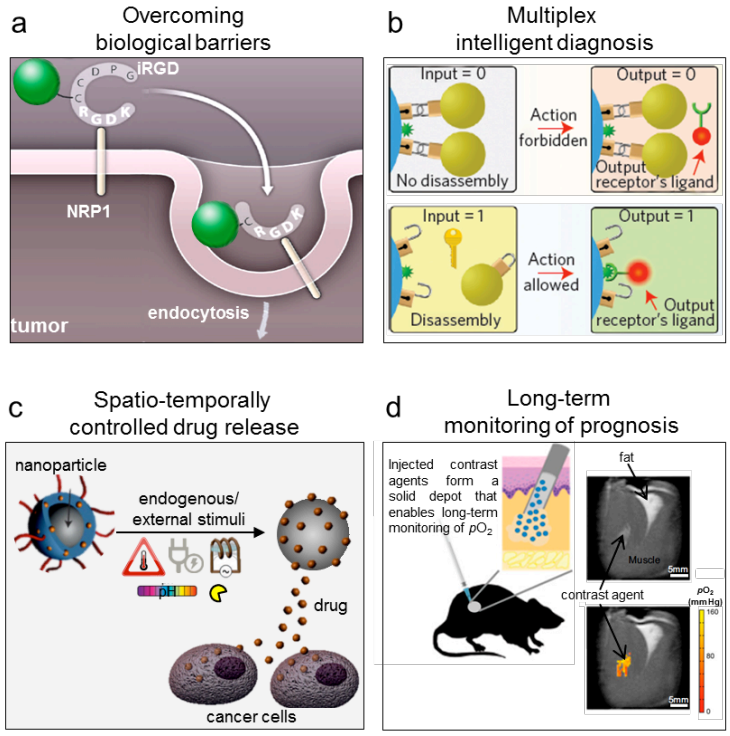

Figure 9. Challenges for future theranostic nanomaterials. (a) Nanomaterials should possess capabilities to overcome hurdles in tumor-specific delivery. One possible approach can be iRGD which allows nanomaterials to access a tumor by penetrating endothelial and tumor tissues. (b) Nanomaterials delivered to tumors should provide comprehensive information about tumor microenvironments. Logic-performing nanomaterials enable smart diagnostics by detecting and processing multiplexed molecular signatures. (c) Based on diagnostic information, nanomaterials should initiate spatio-temporally controlled therapy in response to external or endogenous stimuli. (d) After completing therapy, the non-toxic nanomaterials can be left inside the body and continuously give prognostic information (e.g., oxygen level). (*(a) Reprinted with permission from Feron, 2010; (b) from Nikitin et al., 2014; (c) from Mura et al., 2013; and (d) from Liu et al., 2014*).

Future Challenges

Although current theranostic nanomaterials have great potential, next-generation design concepts and their effective implementation strategies are required (**Figure 9**). Future nanosystems should be able to pass through biological barriers (e.g., BBB, hypoxic tumor regions, stroma, etc.) to reach any tumor sites of the body. One possible approach can be integrating nanomaterials with functional

peptides (i.e., tumor-penetrating peptides) which allow the nanomaterials to reach deep inside an extravascular tumor[146,147]. Magnetic targeting might be another potential solution if the magnetic force exerted on the nanomaterials can be made strong enough to overcome the drag force of blood flow[148,149]. This requires precise control of the direction and intensity of the applied external magnetic field.

When the theranostic nanomaterials arrive at the target site, they should provide quantitative and comprehensive information on the multiple molecular signatures of cancer cells. Current single target-specific imaging and qualitative sensing are not adequate for accurate diagnosis because tumorous environments are complex and heterogeneous[150]. Therefore, nanomaterials should be developed to have multiplexing and logic capability that detects numerous molecular signatures and intelligently reports them to us for accurate diagnostic results[151]. Considering the expression level of those signatures, such diagnostic nanomaterials should possess high sensitivity (e.g., at least pico-molar) for cancer-related biomolecule detection[126].

The nanomaterials have to be designed to sensitively and precisely respond to the corresponding stimuli.

After the diagnosis, spatio-temporally controlled therapeutic action should only start upon reaching the target region in order to lessen collateral damage. The remote trigger of the action can be either multiple and logical combinations of endogenous tumor microenvironments (e.g., pH and enzymes), or exogenously controlled physical stimuli (e.g., light and electromagnetic field)[152,153]. The nanomaterials have to be designed to sensitively and precisely respond to the corresponding stimuli. Simultaneous or sequential execution of therapeutic methods from one nanomaterial also needs to be pursued to overcome cancer resistance (e.g., multidrug resistance)[154]. Finally, when the therapy is complete, the remaining nanomaterials need to be able to assess the treatment's efficacy and aid in making a prognosis[155]. They should of course be fully biodegradable or clearable over time, and in order to meet regulatory requirements, their safety should be ensured for prolonged use through investigation of their clearance (e.g., renal and biliary routes, etc.).

Milestones to address these critical areas that researchers should be able to be achieve over the next 5-15 year time frame include many aspects. In the next 5 years, researchers will establish new sets of design principles to control physical, chemical, structural, and biological properties of nanomaterials for improved sensitivity and specificity in tumor microenvironment monitoring, cancer detection, and therapeutic effect; understand

sub-cellular level interactions between nanomaterials and cancer cells for effective tumor targeting; and evaluate the diagnostic and therapeutic effectiveness of developed nanomaterials by employing *in vitro/in vivo* models. Looking further ahead over the next 10 years, researchers will devise nanomaterials that overcome the biological barriers that limit accessibility to tumors; create nanomaterials with optimal circulation time for enhanced tumor accumulation with minimal off-target effects; endow a multiplexing capability to nanomaterials to identify multiple targets for diagnostic imaging/therapy in real-time; verify the ability to reproducibly initiate therapeutic activity only at tumor/cancer cell sites *in vivo*; and determine nanomaterial safety by characterizing biodistribution, PK/PD depending on size, shape, surface chemistry, etc. In 15 years, researchers will have optimized the theranostic properties of nanomaterials, specifically for prevention/early-detection of cancer, monitoring of cancer heterogeneity, and significant increment in therapeutic index; establish nano-regulatory with industries and the FDA; and make several highly effective nanotechnology based imaging and/or therapeutic agents in the late stage of clinical trials or in the market.

Theranostics: Targeted Theranostics in Cancer

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Introduction

The major challenges in the effective treatment of cancer patients are low efficiency in drug delivery and intrinsic drug resistance in highly heterogeneous human tumors[156,157]. Chemotherapy drugs have short blood half-lives and limited amounts of drugs can be delivered into tumors despite high doses of drugs being administrated to patients that cause severe systemic toxicity. Therefore, improvement of drug delivery into tumor cells should be one of the most important strategies for enhancing therapeutic responses in human cancer.

At present, nanoparticle formulated chemotherapy drugs, such as Doxil (liposome encapsulated doxorubicin) and Abraxane (paclitaxel-albumin protein complex), are FDA-approved nanotherapeutic agents for drug delivery into tumors, which utilize the enhanced permeability and retention (EPR) effect mediated by leaking tumor vessels[158–160]. Various non-targeted or targeted liposome and polymeric nanoparticle drug carriers are in preclinical developments and clinical trials[75,161]. Although those nanotherapeutics have shown promising anti-tumor effects and reduction in systemic toxicity in animal tumor models and in cancer patients, lack of novel approaches for timely assessment of efficiency of intratumoral drug delivery and response remains an issue. It is well known that human tumors are heterogeneous in vasculatures, tumor stromal components, and abnormalities of tumor cells, which contribute to significant differences in physical barriers for drug delivery and intrinsic barriers in drug sensitivity. Therefore, effective cancer therapy not only requires new drug delivery approaches, but also personalized evaluation of drug delivery and the subsequent early tumor response, in individual patients, using noninvasive tumor imaging. This 'precision' version of oncology would make it possible to maximize effectiveness of therapeutic agents by selecting the most efficient drug delivery approach while simultaneously minimizing systemic toxicity through timely replacement of ineffective therapeutic agents.

Current advances in the development of multifunctional nanoparticles with the abilities of targeted drug delivery and imaging intratumoral drug accumulation and distribution, i.e., *theranostics*, offer a unique opportunity for the integration of targeted and image-guided cancer therapy using a single nanoparticle platform[162,163]. First, imaging properties allow for

determining whether a cellular target is expressed by tumors and if this targeted approach is able to deliver sufficient nanoparticles into a specific tumor by non-invasive imaging (**Figure 10A**). In so doing, the cancer patients with the highest likelihood of a clinical response to the targeted theranostic nanoparticle can be selected. This is particularly important for patients with tumors, which are not easily accessible for biopsy. To overcome drug resistance, two or more therapeutic agents can be loaded to a single nanoparticle for targeted delivery into tumor cells, simultaneously, to enhance the synergistic effect of the drugs. This approach has clear advantage over conventional combination chemotherapy since drug molecules with different chemical properties vary in their pharmacokinetics, bioavailability, and stability. Encapsulation or conjugation of drugs to theranostic nanoparticles will significantly improve the blood half-lives of drugs, and protect drug molecules from binding to serum proteins and becoming inactivated by enzymes, leading to targeted delivery of large amounts of active drug molecules into tumor cells.

Following systemic delivery, non-invasive imaging modalities, such as MRI, PET, ultrasonic, photoacoustic, and optical imaging, can be used for determining nanoparticle-drug delivery efficiency (**Figure 10B**). Using an imaging modality with high resolution and anatomic information, it is feasible to monitor early tumor responses following targeted therapy to identify imaging signatures that predicate a good or poor response such that ineffective drugs will be replaced with more potent therapeutics in a timely manner (**Figure 10C and D**). Finally, targeted delivery of multimodal imaging theranostic nanoparticles enables intraoperative detection and removal of drug resistant tumors using image-guided surgery (**Figure 10E**).

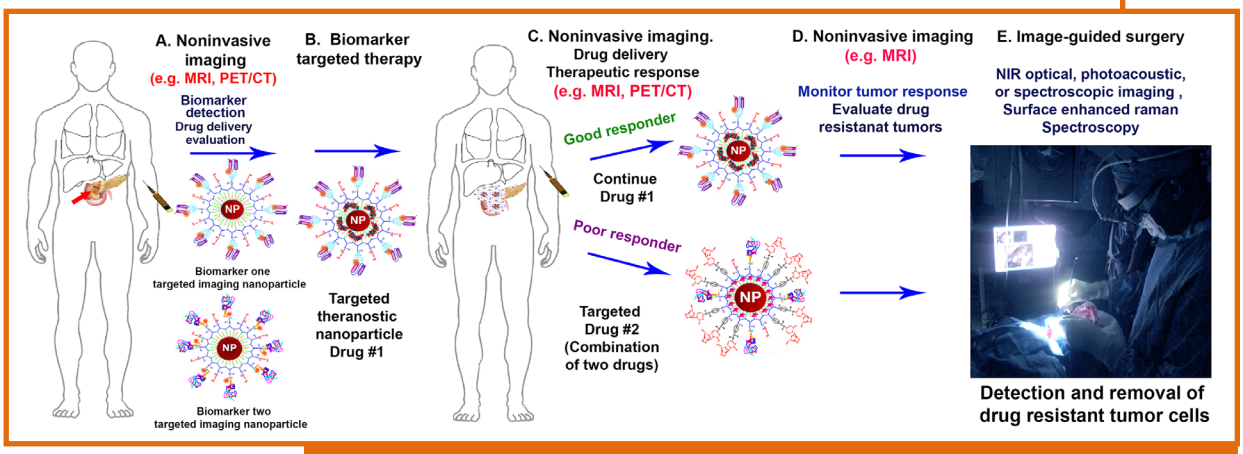

Figure 10. Clinical paradigm for theranostic nanoparticles. An outline of steps [A-E] along the clinical path of which theranostic nanosystems would display their inherent importance in oncology.

The development and translation of image-guided and targeted therapy using theranostic nanoparticles have clinical significance in the treatment of several aggressive cancer types, such as triple negative breast, pancreatic, ovarian, lung, colon, and liver cancers. For example, neoadjuvant chemotherapy has been given to triple negative breast cancer (TNBC) patients before surgery. About 22% of TNBC patients showed a good therapeutic response (pathologic complete response) and an excellent prognosis[164]. TNBC patients with drug resistant tumors following neoadjuvant therapy have a high incidence of tumor recurrence and a poorer survival. Image-guided neoadjuvant therapy using theranostic nanoparticles will allow for the selection of more potent therapeutics for individual patients while reducing systemic toxicity. Additionally, the integration of image-guided and targeted therapy using theranostic nanoparticles offers the possibility of reduction of tumor burdens of un-resectable pancreatic cancers, including over 50% of pancreatic cancer patients with locally advanced diseases[165], for potentially curative surgery. Optical image-guided surgery enables for complete removal of drug resistant tumors in those patients. Therefore, success in the development of targeted theranostic nanoparticles and innovative imaging approaches has the potential to change the paradigm of future clinical management of cancer patients.

The importance of theranostics in cancer therapy has promoted rapid advances in the development of various types of theranostic nanoparticles.

Current State of the Art

The importance of theranostics in cancer therapy has promoted rapid advances in the development of various types of theranostic nanoparticles. However, challenges in the development of such a class of multifunctional nanoparticles are well recognized. As a drug carrier, it is necessary to select nanomaterials that are biodegradable with low toxicity even after repeated administrations at high doses. It requires high drug loading and conditional drug release in tumor cells. Production of strong and lasting imaging signals is also required. Active targeting to cell surface receptors highly expressed in tumor cells is critical for increasing not only drug delivery into tumor tissues, but also into tumor cells by endocytosis. Theranostic nanoparticles targeting multiple cell types in the tumor, such as tumor endothelial cells, stromal fibroblasts and macrophages, and tumor cells have been shown to enhance intratumoral delivery of targeted nanoparticles[166]. Examples of the cellular receptors that are highly expressed in tumor stromal and tumor cells are uPAR, IGF-1R, folate receptor, and integrin $\alpha v\beta 3$. Several examples of cellular receptors that are highly expressed in tumor cells include EGFR, HER2, MUC1, and CEA.

Theranostic nanoparticles have been produced by conjugation and encapsulation of radiotracers to nanoparticles for PET imaging or gadolinium for MRI[167]. Those approaches are used for converting liposomal, polymeric, silica, and dendrimer nanoparticles into theranostic agents. PET/CT detects targeted delivery of radioisotope labeled nanoparticles with high sensitivity. However, repeated administrations of large amounts of radioactive agents and exposure to high doses of ionizing radiation in combination with CT imaging are the major concerns. Relatively short half-lives of radioisotopes require the theranostic nanoparticles to be administrated into the patients in a short time after labeling with radiotracers. This also makes it difficult to monitor therapeutic responses, which often take days or weeks.

Near infrared (NIR) fluorescent dye conjugated or encapsulated nanoparticles are promising optical imaging probes for image-guided surgery, which represents another theranostic application. The effect of pH-sensitive or protease-activated polymeric nanoparticles carrying NIR dyes on identification of tumor margins for surgical resection has been demonstrated in animal tumor models[168,169]. Results from a recent clinical trial using RGD peptide conjugated ultra-small fluorescent silica nanoparticles labeled with a radiotracer (iodine) showed that it is safe for systemic administration in human melanoma patients and the nanoparticles were cleared through renal excretion[28].

Metallic magnetic iron oxide and gold nanoparticles are commonly used theranostic nanoparticle platforms in preclinical studies. Biodegradable magnetic iron oxide nanoparticle (IONP) with MRI contrast is one of the most promising theranostic nanoparticles for clinical translation. Therapeutic agents are conjugated to or encapsulated in the surface coating of the nanoparticles. Targeted theranostic IONPs have been developed and their effects on tumor growth and MRI of nanoparticle-drug delivery have been demonstrated in preclinical studies[170–172]. In comparison with other imaging modalities, MRI has imaging depth and high-resolution 3D-imaging capability for interrogation of heterogeneous intratumoral drug distribution. IONPs can serve as both T_1 and T_2 contrast agents depending on the core sizes and MRI scan methods[173–175]. IONPs are relatively stable in the tumor for an appropriate length of time for monitoring tumor responses to therapy by MRI. In combination with clinical contrast enhanced MRI imaging signatures of the early tumor response may be identified. A drawback of MRI is relatively high costs. Further

...MRI has imaging depth and high-resolution 3D-imaging capability for interrogation of heterogeneous intratumoral drug distribution.

improvements of T_1-contrast imaging approaches should increase sensitivity and specificity of detecting small tumor lesions in organs with a low MRI contrast, such as the liver and lung. Targeted IONPs conjugated with NIR dyes can be used for intraoperative detection of drug resistant tumors[166,176].

Theranostic applications of gold nanoparticles have been developed[77,177]. Targeted delivery of gold nanoparticles generates plasmonic photothermal bubbles that promote drug release from nanoparticle drug carriers in the endosome of cells[178]. Although gold-based theranostic nanoparticles have been produced and tested in animal tumor models, there is a concern about its low biodegradability and lack of a well-defined mechanism of clearance following systemic delivery in large therapeutic doses.

A multi-spectral imaging approach using a Raman endoscopic imaging device and tumor targeted surface-enhanced Raman scattering (SERS) gold nanoparticles has been developed for cancer detection and image-guided resection. Feasibility of multiplexed tumor imaging using SERS has been demonstrated in animal tumor models and in excised human colon tissues[179]. Image-guided hyperthermia treatment using NIR signals produced by photosensitizing agents conjugated to metallic nanoparticles has also been tested in animal tumor models[180]. Accumulation of the nanoparticles in tumors allows for image-guided therapy by precisely applying a laser to the tumor sites.

Future Science and Clinical Development

Clinical development of theranostic nanoparticles has to address challenges that are common for all cancer therapeutics and nanoparticle drug delivery systems as well as unique requirements for its dual therapeutic and imaging applications. Research areas that may have the most impact on clinical translations includes: (1) Development of ultra-small and biodegradable nanomaterials with high imaging signal strengths, high drug loading capacity, and conditional drug release ability; (2) Innovative targeting approaches and nanoparticle designs that significantly enhance passive and active targeting for intratumoral drug delivery, avoid non-specific uptake by macrophages, and have the ability of overcoming tumor stromal barrier for improving drug delivery into tumor cells; (3) Combined delivery of potent therapeutic agents for the treatment of drug resistant tumors; and (4) understanding mechanisms of nanoparticle-drug delivery and interactions of targeted theranostic nanoparticles with tumor cells and tumor microenvironment in animal tumor models that are highly relevant to human cancers, such as human patient tissue derived xenograft (PDX) tumor models and transgenic mouse tumor models. Finally, large-scale production of Good Manufacturing Practices grade theranostic nanoparticles for human use will be the major challenge. It requires the production of consistent nanoparticle core and coating, efficiency

in drug loading, and conjugation of large amounts of endotoxin-free and bioactive targeting ligands to the nanoparticles.

With the joint efforts of the NCI Alliance of Nanotechnology for Cancer and investigators at academic institutes and within industry, several advances should come to fruition over the upcoming 5-15 year time frame. In the next 5 years, researchers will complete preclinical studies for 5 to 6 targeted theranostic nanoparticle platforms; File IND applications for 3 to 4 of the above nanoparticles for Phase I clinical trials; and begin 2 phase I clinical trials for image-guided surgery using targeted imaging nanoprobes. Looking further ahead over the next 10 years, researchers will generate 3 to 4 new theranostic nanoparticles and image-guided cancer therapy protocols in Phase 1 clinical trials; 1 to 2 Phase II/III clinical trials using an integrated image-guided and targeted therapeutic clinical protocol for personalized cancer treatment; and receive FDA approval of 1 targeted imaging nanoparticle for image-guided surgery. Even further out over the next 15 years, researchers will complete 1 to 2 Phase 11/III trials; gain FDA approval of 1 theranostic nanoparticle and associated image-guided therapy protocol; and initiate 5 to 6 new clinical trials using theranostic nanoparticles and image-guided treatment protocols.

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