

Keys and regulators of nanoscale theranostics

Amineh Ghaderi^{1,§}, Eduardo Antunez de Mayolo^{2,§}, Hirak Kumar Patra^{1,§}, Mohsen Golabi^{1,§}, Onur Parlak^{1,§}, Rickard Gunnarsson^{3,§}, Raul Campos^{4,§}, Revuri Vishnu^{1,§}, Sami Elhag^{5,§}, Selvakumar Subramanain^{1,§}, Wetra Yandi^{6,§}, Yuan Liu^{4,§}, Yugal Agrawal^{1,§} and Ashutosh Tiwari^{1,7,*}

¹Biosensors and Bioelectronics Centre, IFM, Linköping University, Linköping 583 81, Sweden

²Institute of Experimental Physics, Otto-von-Guericke University Magdeburg, Germany

³Plasma & Coatings Physics Division, IFM-Materials, Physics, Linköping University, SE-581 83 Linköping, Sweden

⁴Department of Chemistry, IFM, Linköping University, Linköping 583 81, Sweden

⁵Department of Science and Technology, Campus Norrköping, Linköping University, Norrköping 60174, Sweden

⁶Molecular Physics, IFM, Linköping University, Linköping 583 81, Sweden

⁷Tekidag AB, Linköping 58431, Sweden

§ Authors contributed equally and all names arranged in alphabetical order.

*Corresponding author. E-mail: ashutosh.tiwari@liu.se; Tel: (+46) 13282395; Fax: (+46) 13137568

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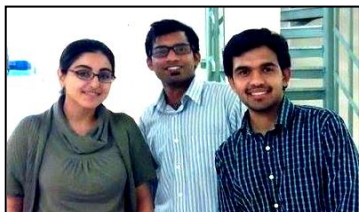
ABSTRACT

Nanoscale theragnosis is the biomedical aspect of nanomaterials for simultaneous diagnosis and therapy. The last decade was completely devoted by the scientist to combine the advancement in nanotechnology molecular biotechnology for the development of future nanomedicine. The approach started with the development of target-specific delivery of the cargo imaging molecule or drugs for biomedical applications. The cutting edge advantages of the nanoscale materials (e.g., large surface to volume ratio, size-shape dependent physicochemical properties and multi-functionality etc) proved themselves as the most potential preferences to design optimal therapy for the personalized medicine. The present tutorial review will highlight the recent advances in the development on the regulation of such theragnosis system and their biomedical perspectives to act as a future nanomedicine. Copyright © 2015 VBRI Press.

Keywords: Nanomedicine; theranostics; therapy; personalized medicine.



Left to right: Mohsen Golabi, Sami Elhag, Eduardo Antunez de Mayolo, Yuan Liu, Selvakumar Subramanain, Raul Campos, Onur Parlak, Hirak Kumar Patra, Rickard Gunnarsson, Wetra Yandi, and Ashutosh Tiwari;



Left to right: Amineh Ghaderi, Yugal Agrawal and Revuri Vishnu.

Introduction

In the growing number of fatalities resulting from numerous diseases including cancer, diabetes and other diseases that eventually cause the worst health illness possibly and leads to death; new enabling tools are required to provide extensive molecular profiles of patients to guide the clinician in making viable diagnosis and prognosis. As the recent developments in academics and medical centers introduced point-of-care application for diagnostic and therapeutics and persons separate data accounts, maintain for all time use as personalized medicine (PM) [1-2]. Furthermore, nanotechnology makes nano-objects as a part in the current technology, and was introduced after a famous speech of Richard Feynman to the American physical society, Pasadena in 1959 where he was explored the possibilities afforded by miniaturization.

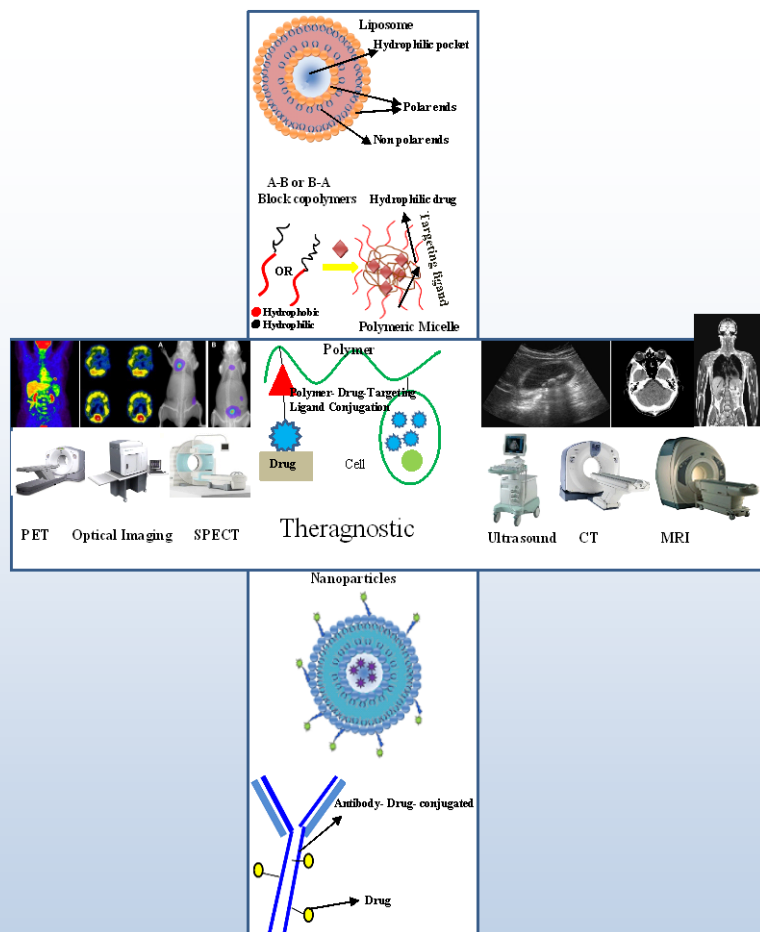


Fig. 1. Concept of theranostics includes therapeutic part in the vertical column and diagnostic part in the horizontal column. In therapeutic part different ways of therapy methods such as liposome, polymeric micelle, polymer-drug-targeting ligand conjugation, nanoparticle and antibody-drug-conjugated are indicated. In diagnostic part different ways of diagnostic like positron emission tomography (PET), optical imaging, and single-photon emission computed tomography (SPECT), ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI) have been shown.

During past two decades rapid growth in nanotechnology has increased tremendously and had opened doors for many potential applications including foods, cosmetics, consumer goods, military, vehicles manufacturers, and biomedical applications [3]. In biomedical sciences, a range of bioengineered nanomaterials have been studied and under in depth investigation to be used in targeted drug delivery, real-time patient monitoring, biosensing, and high resolution non-invasive bio imaging, high throughput screening and multiplexed diagnostic system [4]. The advanced studies on application of nanomaterials in biomedical have demonstrated that nanomaterials can act not only as diagnostic and imaging agents but also as therapeutic or therapy-guided agents [5]. The concept of theragnostic has emerged as the means to this end. Theragnostic is the combination of diagnostics and therapeutics as introduced by Pharma Netics in 2002, and described as a rapid diagnostics and facilitating the selection of the right drug, in the right dose, at the right moment for the physicians' therapy. Since then, considerable efforts have been devoted to functionalize nanoparticles such as iron, gold, graphene etc. by numerous molecular and polymeric materials [6-8].

Nanomaterials are now being bioengineered for efficient transport, capable of electron and optical excitation in addition to biosafety and biocompatibility. The physicochemical aspects of the nanostructures are promising for such applications due to higher surface-to-volume ratio, very low limit of detection, and the possibility to fabricate point-of-care diagnostic devices [9]. The metallic nanoparticles such as gold and silver are often considered as the effective labels for their higher sensitivity in optical detection-based methods such as surface plasmon resonance (SPR) that leads them to absorb NIR light and convert it into vibrational energy (heat) which not only open the possibility of absorption spectroscopy but also offer prospective in photo thermal therapy (PTT). The specialized optical properties of quantum dots and carbon nanotubes are the other modules served as labels for in vitro molecular diagnostics. The bioengineered nanomaterials showed potential impact on in vivo imaging in diagnostic visualization of targeted cells and/or molecules through positron emission tomography (PET) and single photon emission computed tomography (SPECT), X-ray computed tomography (CT), magnetic resonance imaging (MRI), optical imaging, and ultrasound imaging (US imaging). Nanomaterials can serve as vehicle for radioactive contrast agent in PET and SPECT to manipulate the behavior of nuclear isotopes in the biological environment. The paramagnetic metallic complex nanoparticles, such as gadolinium and iron oxide have been tested as contrast agents in MRI whereas polymeric nanoparticles can generate nano bubbles for US imaging in vivo. Apart from these label-free techniques quantum dots (QDs) and gold-based nanostructures have been used to enhance bioluminescence and fluorescent contrast in vivo.

The development of bioengineered smart nanomaterials to respond the disease hallmark function is the recent arena of nanomedicine. It is a newly emerging supra-disciplinary field with growing clinical potentials. Stimuli-responsive nanobiosystems answer by a considerable change in their properties to small changes with disease progressions for example i) physical-temperature, electric or magnetic fields, light and mechanical stress; ii) chemical - pH, ionic factors and iii) biological factors - enzymes and biomolecules. Such responsive biosystems are attractive and increasingly more prevalent as scientists learn about the chemistry and triggers that induce conformational changes in structures and devise ways to take advantage of and control them inside or outside the body. New smart bioengineered nanosystems are being formulated that sense specific environmental changes and adjust in a predictable manner, making them useful bio-tools. The progress in this field would make significant contributions to advanced medical technology, bioelectronics, nanomaterials and

nanotechnology. The aim of this tutorial article is to discuss various strategies of cutting-edge bioengineered nanosystems for biomedical devices.

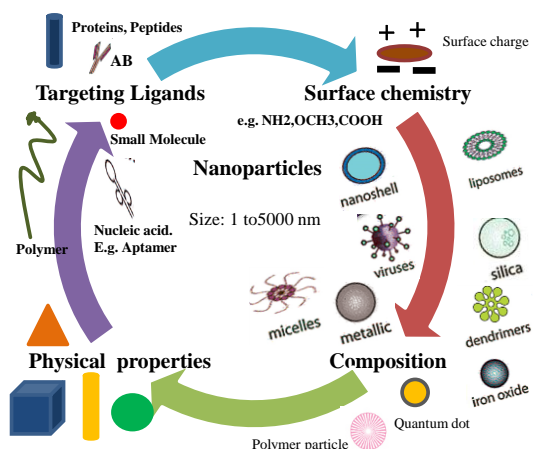


Fig. 2. The properties of nanoparticles which can be assembled from different chemical ($-NH_2$, $-OCH_3$, $-COOH$ and surface charge) and physical properties (triangle, cubes, sphere and rods) and different materials composition (Nano shell, liposomes, viruses, silica, metallic, micelles, quantum dots, dendrimers, iron oxide and polymer particle) as well as numerous ligands (e.g., proteins, peptides, polymer, aptamer and small molecule) for biological targeting.

In vitro theranostics

In vitro theranostic is a kind of diagnostic and therapeutic test that are performed to detect diseases in an artificial environment outside the living body with their simultaneous response to the drug to be administered, which are usually carried out in test tubes in a laboratory. For example, Patra et al has developed nano architecture with stimuli-responsive nano theragnostic platform for specific targeting, controlled release of the drug into tumour sites with real time monitoring capability through Magnetic Resonance [10]. *In vitro* theragnostic tests are expected to provide better and accurate detection of different diseases conditions and thus it will help to establish an appropriate treatment to the patients. Currently, to detect different kinds of diseases, a variety of *in vitro* diagnostic tests are employed such as immunoassay, molecular diagnostics, hematology, flow cytometry, coagulation and clinical chemistry. Among them, molecular diagnostics is growing rapidly [11]. A brief description of such systems that might be integrated in future theragnostic module described below.

Molecular genetic diagnostic test is operated through detecting specific sequences in DNA or RNA that may or may not be associated with disease, including single nucleotide polymorphism (SNP), deletions, rearrangements, insertions and others; [12-13] nowadays, these tests are an integral part of medicine, available for a large number of human disorders, including single gene diseases, polygenic diseases, and chromosomal syndromes [14] Three basic steps are included in the molecular assay: the extraction and purification of nucleic acid; the amplification or making copies of the nucleic acid of interest or attaching multiple copies of a dye to a single target copy; the detection of the DNA can be amplified target using real time polymerase chain reaction (PCR) or end product detection including

microarrays, Luminex (similar to flow cytometry), or sequencing [12]. Many different methods of DNA analysis have been described and applied, however, most of which are based on amplification of the DNA region of interest by PCR. PCR with a number of its modifications has been the golden standard in molecular genetic testing for more than twenty years, in which real time PCR can provide elegant and effective quantification or genotyping of the tested material [14]. As a powerful diagnostic tool, molecular genetic diagnostic have been carried out in many studies. Meder et al. presented an approach for molecular genetic diagnostic of cardiomyopathies using targeted next-generation sequencing, which can genetically characterize patients in a fast, comprehensive, and cost-efficient manner. [15] Furthermore, the developments of molecular genetic diagnostic in epilepsy are summarized, and the general approach to genetic evaluations of epilepsy is presented [16]. In addition, several researchers give a simple introduction of molecular genetic diagnostic from Serbia, and the genetic testing in thrombophilia is reported [14].

Molecular genetic diagnostic is a powerful and effective tool in disease diagnostic and the exploration of pathogenesis. As reported, inherited cardiomyopathies can be caused by mutations in more than thirty different genes. [15]. It is hardly possible to genetically characterize patients with cardiomyopathies in a fast, comprehensive, and cost-efficient way. However, recently an approach is established through an array-based sub genomic enrichment followed by next-generation sequencing, as shown in (Scheme required). Using this method, the genomic region of interest can be enriched by a mean factor of 2169 compared with the coverage of the whole genome, which results in high sequence coverage of selected disease genes. The disease-causing mutations can be detected in patients. Then, the genetic pathogenesis of cardiomyopathies can be revealed. Furthermore, genetically characterizing patients with cardiomyopathy is allowed to be done for the first time in a fast, comprehensive, and cost-efficient manner [15].

Real-time theranostics

New theragnostic applications of bioengineered nanomaterials promise a future where administered drugs can be followed and their efficacy monitored real time in the human body through real time imaging. Potential applications of theragnostic nanomedicine formulations range from the noninvasive assessment of therapeutic agent biodistribution at the target site, monitor and quantify drug release, facilitate therapeutic intervention using triggered drug release and longitudinally monitor the efficacy of therapeutic interventions [17]. By labeling drugs with bio imaging contrast agents, such as radionuclides and MR imaging probes, the drug distribution can be visualized. This has benefits in both the experimental study of the drugs interaction with the host as well as clinical benefits as it allows seeing how well the patient responds to the drug. Another possibility is to trigger and image the effective release of the drug specifically at the site of interest that has profound benefits in cancer therapy. Moreover, if the imaging agents stay at the tumor area for longer periods of time, a continuous size diagnosis can be made [17].

Table 1. Theranostic imaging: Representative nano-carrier hybrid systems for single/multi-modality tumor imaging.

Imaging Mode	NPs	Polymers Used	Drug	Dye/Radionuclide/Chelating agent	Targeted Molecule	Ref.
MRI	SPION	PAA	Taxol	DII, DIR	Folic acid Ligand	[20]
	IONP	Oleic acid /pluronic F127	Taxol/DOx	-	-	[21]
	MNPs	Poly(lactide-co-glycolide (PLGA)	Hydrophobic : philic (1:2)	-	Herceptin	[22]
	Reduced Graphene oxide -IOP	PEG	-	-	-	[23]
	Manganese doped-IOP	Bovin Serum Albumin(BS A)/PEG	-	-	RGD Peptide/siRNA	[24]
	SPION	Poly(lactico-glycolic acid	Dtxl	-	scAb(PSCA)	[25]
CT	Au NPs	-	DOx	-	PSMA RNA Aptamer	[26]
NIRF	Polymeric NPs	-	-	Cathepsin B & MMP	-	[27]
	Au NPs	-	-	AlPcS(4)	-	[28]
	PEG-b-PLA(Polymeric Micelle)	-	PTX/17-AAG/RAPA	-	-	[29]
	PEG-b-poly(ϵ -caprolactone) (PEG-b-PCL)	-	-	DIR	-	[29]
Fluorescence	Au Nanorods	-	-	MMP2	-	[30]
Optical	Chitosan NPs	-	-	Cy5.5	-	[31]
	SPION	PAA	Taxol	DII, DIR	Folic acid Ligand	[20]
	Hyaluronic acid NPs	Pegylation	-	Cy5.5	-	[32]
	MNPs	Oleic acid & pluronic block Co-polymers	-	Dyes(5700,6825, 5491, 5177 & 2826)	-	[33]
PET/MRI	IOPs	Poly-Aspartic acid(PASP)	-	DOTA	RGD Peptides	[34]
PET/NIRF	QDs(Amine Functionalised)	-	-	DOTA,Cu-64	RGD Peptides	[35]
	Ferritin Nanocages	-	-	Cy5.5, Cu-64	RGD Peptides Anti-Her2 Antibody	[36]
CT/MRI	Fe-Pt NPs	-	-	-	-	[37]
	IO/Au Hybrid NPs	Poly(DMA-r-mPEGMA)	-	-	-	[38]
MRI/SPECT	SPION	-	-	-	antimesothelin antibody mAbMB	[39]
Optical/SPECT	PEG Coated Micelles	-	-	In-111	Annexin-A5	[40]
MRI/NIRF	SPION	-	-	Cy5.5	EPPT Peptide, siRNA	[41]
	IONP	PEG-g-chitosan Co-polymer	-	Cy5.5	Chlorotoxin (CTX)	[42]
SPECT/CT	Poly(N-vinylimidazole-co-N-vinylpyrrolidone)- β -p(d,l-lactide) & PEG-PLA di-block polymers	-	DOx	-	Folic acid	[43]
Optical/MRI/PET	TCL-SPION	-	-	I-124	-	[44]
	Er(3+)/Yb(3+) co-doped NaGdF(4) Nanospheres	-	-	I-124	-	[45]
Optical/SPECT/MRI	Cobalt Ferrite NP	-	EDC	Ga-67	-	[46]
Optical/PET/NIRF	IONP	Human Serum Albumin(HSA)	-	DOTA, Cu-64 & Cy5.5	-	[47]

One emerging imaging technique that has not yet made a breakthrough clinically is magnetic particle imaging (MPI). MPI utilizes oscillating magnetic fields to detect super paramagnetic nanoparticles that are dispersed in a biological system, having more sensitivity than magnetic resonance imaging (MRI). Another benefit of MPI is that it does not utilize ionizing radiation. Hence, this technique has the potential to be used for clinical theranostic applications. For example, MPI could be used as a diagnosis tool for cardiovascular disease; the super paramagnetic particles can be intravenously injected in the patient to monitor cardiovascular activity in real time.

Therefore it could be used to see blockages in the circulatory system or to monitor a balloon catheter while expanding obstructed arteries. To cite another example, during stem cell therapy is hard to track the localization of the therapeutic cells; by labeling them with super paramagnetic nanoparticles, MPI can be used to image cell dispersion in real time. MPI can also be used in cancer therapy to monitor blood flow to tumors. If the super paramagnetic nanoparticles are functionalized to target tumors, an alternating magnetic field can potentially locally heat up the area of high particle concentration and eradicate the tumor [18].

Different imaging methods have different advantages and disadvantages. For instance positron emission tomography (PET) and MPI are both sensitive techniques but do not give any information about the anatomical background. Therefore by combining different imaging methods in one, the disadvantages can be reduced. To achieve this, the contrast agent has to be specially engineered to respond to both imaging methods. By putting radio nuclides on gold nanoparticles, the area of interest can both be seen by PET and Computed Tomography (CT) which gives the necessary anatomical background. By adding a fluorescent molecule to iron oxide nanoparticles that are capable of targeting cancer cells, the tumor can first be imaged by MRI to plan an operation. Then, during the surgical procedure, the tumor can be imaged and distinguished from healthy tissue using fluorescence imaging [19].

Different treatments can also be combined to enhance therapeutic effects. Q. Xiao et al. [48] synthesized silica-coated rare earth core-shell nanoparticles with smaller copper particles attached on the shell. These particles were both responsive to near infrared light and X-ray radiation making it possible to combine photo thermal ablation with radiotherapy. They were also suitable for imaging methods such as up conversion luminescence, CT and MRI [48].

An elegant example of a formulation that facilitates imaging of correct target-site accumulation and triggered drug release was developed by Langereis et al. [49]. They created thermo-sensitive liposomes containing two different MR contrast agents; a ^1H -labeled agent and a ^{19}F probe (drug), both located in the aqueous interior of the liposome. Only the ^1H -labeled agent can be seen as long as the liposomes are intact. Upon heating to temperatures above 38°C , which can be done locally, the liposome releases the content and only the ^{19}F probe can be imaged (need a scheme) [49]. Image guided therapy advances quickly and its role in pursuing improved therapeutic treatments is further encouraged by the recent outstanding results previously presented. In the future, scientists can easily imagine that theranostics will have an important role in the development of personalized medicine. With this scope, more work is today necessary to develop systems with more target specificity and more effective drug-release responsiveness.

Deep inside with theranostic modules

Although in vivo imaging starts from van Leeuwenhoek studies and Brownian motion observation, it was the recent development of new imaging modalities that cause notably

progress in this field to help us extracting quantitative information in the living animal [50]. Tracing biomolecules in the natural environment by using of *in vivo* molecular imaging techniques has the potential to reveal bimolecular interaction processes and metabolic pathways. Bio-imaging and visualization of specific subsets of cells is broadly used in immunological research. So, advanced *in-vivo* molecular imaging techniques are expected to be pivotal for many research, diagnostic, and therapeutic base applications such as early stage cancer diagnostics, understanding the fundamental molecular pathways, guided stem cell therapy, image guided surgery, cancer staging, drug delivery and gene therapy [51].

The most routine modalities using in the *in vivo* imaging research field include near-infrared fluorescence imaging (NIRF), magnetic resonance (MRI), computed tomography (CT), ultrasound (US), and positron emission tomography (PET). Specific priority and intrinsic limitations such as sensitivity and spatial resolution are supposed for each modality. Most of biomolecules and intracellular structures are transparent. So, various types of probes have been widely used for *in vivo* molecular imaging. Fluorescent dyes, synthetic aromatic chemicals, are routinely used to stain cells for better visibility. These probes bind with biomolecules or specifically localize in a structural region. Although fluorescent dyes broadly apply in this field, the main problem is irreversible damage to the probe due to extend exposure to the excitation light source caused interrupted the observation.

However nanoparticle-based contrast agents are not without issues, they have the potential to improve the quality of medical diagnostics for a wide range of *in-vivo* imaging modalities and potentially enhance targeting efficiencies via longer engineered circulation times, designed clearance pathways, and multiple binding capacities in comparison with molecular scale based contrast agents [52]. Quantum Dots (QDs), nanocrystal semiconductor material, have been successfully applied as tracking tags on antibodies, proteins and DNAs for prolonged bio-imaging purposes [53]. UV excitation creates whole electron pairs in the QDs nano-crystal and narrow spectra in visible light range, due to the nanoparticle size, emits when the electron pairs recombine. A new class of materials, being developed as agents for *in-vivo* imaging, is up-converting NPs, doped with rare-earth ions, which absorb NIR light and emit up-converted light at a higher energy [54].

To achieve high tissue contrast and improve imaging sensitivity, NP-based probes have been developed for MRI. Iron oxide NPs, generally coated with dextran, PEG, or other polymers are the most popular material studied for using in clinical MRI [55].

PET is an imaging technique based on radioisotopes emission without the need for external excitation. There is a potential to combine other modalities include NIRF or MRI with PET [26]. X-ray has been broadly using in the field of bio-imaging but little attempt was devoted for expanding of nanomaterial based contrast agents for this modality. The number of research on the fate, transport, and toxicology of gold-based NPs revealed the great potential of using them as a promising next generation candidate for X-ray contrast materials [56]. NPs consisting of bismuth sulfide,

composite ceramics containing iron oxide and lanthanide materials have been reported as contrast agents in X-ray imaging technique [57,58]. The combination of image modalities and development of nanoparticle-based contrast agents are potential ways to boost the resolution and sensitivity of *in vivo* molecular imaging.

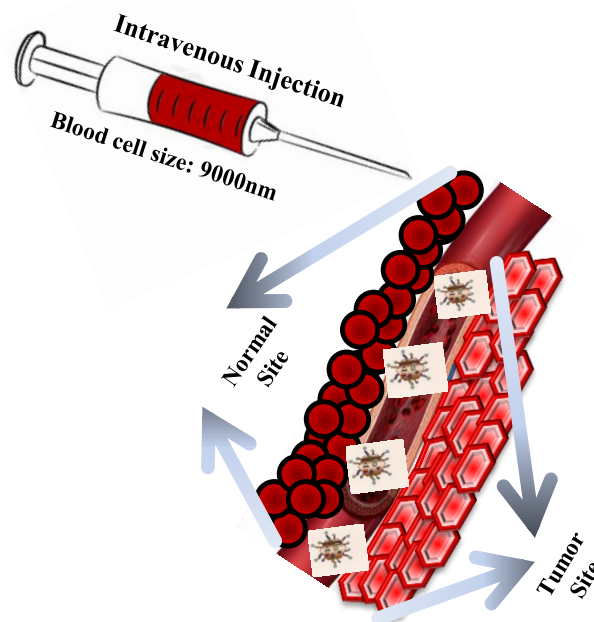


Fig. 3. As smart nanoparticles injected to the vein they are go through the cancerous site of the tissue and enter into the cancer cell.

Theranostic regulator

Responsive Nanomaterials have received prodigious attention especially in the field of nanomedicine. They could be defined as dynamic materials that can acknowledge the surroundings and counteract by transforming their physical characteristics and produce significant effects [59]. Nanocarrier based drug delivery systems are advantageous over conventional systems as they facilitate multiple surface functionalizing, exalt image resolution, multiple drug targeting, enhanced circulation time, improved therapeutic efficiency and reduced toxicity [60]. Usage of Smart stimuli responsive nanosystems has evolved a dimension as they are successful in pro-drug based site specific targeting that could prolong the drug release by which the pharmacokinetics of the drugs can be tailored [61]. These Nanoscale material can respond to both Exogenous stimuli that includes Temperature, Heat, Light, Ultrasound, Magnetic field and Endogenous stimuli like pH, Redox, Enzymes and molecular analytes. Due to the tremendous work in the field of materials science, a broad range of nanomaterials with multiple sizes, geometry and physical properties have been established. They include liposomes, micelles, polymeric materials, gold nanoparticles, Iron-oxide nanoparticles, cyclodextrins, dendrimers, mesoporous silica nanoparticles and a variety of organic and Inorganic materials. Here in we would discuss about different stimuli responsive intelligent nanocarrier systems, emphasize on their mechanisms of action and performed actions.

Temperature

Temperature responsive nanomaterial systems have received a tremendous significance in the contemporary field of research. A nonlinear acute change in the property of the material on exposure to temperature is the fundamental strategy of the thermo responsive systems. As a rule, thermo responsive systems should not release the cargo at body temperatures and should respond to a temperature about 40-42°C, which is the native tumor's temperature. So far Liposomes and polymeric materials have been extensively studied as Thermo responsive drug delivery systems.

Thermo sensitive Liposomes response is based on the phase transition changes in the lipid constitutions that enhance the fluidization and motility of lipids creating gaps and propelling drug release. Widely used lipids for the construction of thermo sensitive Liposomes are DPPC (Dipalmitoylphosphatidylcholine) [62]. Surface functionalizing these liposomes with PEG (Poly Ethylene Glycol) can enhance the blood circulation time. Poly ethylene glycol facilitates stealth characteristics to the nanocarriers as they prevent the adsorption of opsonizing proteins and activation of immune systems that in turn enhances the blood circulation time [63]. Other strategies include thermo responsive, gated drug delivery systems. Leucine Zipper, an amphiphilic peptide can dissociate into disordered confirmation above its melting point (40°C) has been incorporated into the Liposomes for the gated drug release [64]. Thermo responsive liposomes have been leading thermo responsive systems in terms of clinical trials. Drug like Doxorubicin in association with hyperthermia active materials have been loaded into Liposomes (Thermo DOx, Celsion Corporation) and are presently in Phase II of the clinical trials to treat Breast and colorectal cancers and Phase III in case of hepatocellular carcinoma.

Generally used polymeric materials as a thermo responsive materials would be Poly (N-isopropylacrylamide) (PNIPAM). PNIPAM is a Lower critical solution temperature (LCST) polymer which can transform from hydrophilic phase (<32°C) to hydrophobic phase upon raise in temperature (>32°C). When the temperature is less than 32°C, PNIPAM is believed to have intermolecular hydrogen bonding with the surrounding water molecules. When the temperature is raised above 32°C, the hydrogen bondings with the surrounding water molecules breaks down and due to the entropy constrains, the hydrophobic forces enact to have an association with the neighboring polymer chains leading to a collapse of the polymer chains which tries to minimize contact with water molecules [65]. CST of PNIPAM is independent of molecular weight or concentration but depends on hydrophilic/hydrophobic balance. Myriad numbers of formulations have been strategized based on PNIPAM and PNIPAM modified structures. They have also been explored in tissue engineering as an artificial extracellular matrix. Other polymeric materials used as thermo responsive a material includes, Poly (methyl vinyl ether) PMVE, Poly (N-vinyl caprolactam) PVCA, Poly (N-ethyl oxazoline) PEtOx, Poly (acrylic acid-co-acrylamide) and many more. Most of them were unsuccessful as their CSTs were incompatible with human body temperatures [66].

Photosensitive

Due to their promising noninvasiveness and their ability to remote spatiotemporal control, a plethora of drug delivery systems have been constructed based on photoactivated systems. Illumination with a specific wavelength (UV, Visible or Infrared regions), an ignition in drug release could be achieved. They can be tuned either one time burst release or switchable (On/Off) controlled release based on the structural modifications in the nanocarrier. One of their applications includes, Azobenzene modified Mesoporous silica nanoparticles. Azobenzene can photoisomerize from their native Trans form to cis form upon irradiation with a light of wavelength 300-380 nm. Interestingly, they can switch back to their native trans form upon their exposure to visible light. These photoswitchable structures incorporated in pores of the mesoporous silica nanoparticles reduced their size upon the transition, enabling controlled and gated drug delivery [67].

One of the major disadvantages of using light with a wavelength in UV region is their low penetration depth (~10 mm) due to the high scattering of soft tissues in UV region. Thus their application is limited to topical drug delivery. However this hurdle was surmounted by using molecules that can respond to light in NIR region. Recent advancements include application of Upconversion (UC) nanoparticles for tunable drug delivery. UC process depends on the multiple excited energy levels that can accumulate photons excited at lower energy. This process depends on ensuing absorption of two or more photons of lower energy excitation within an energy level that subsequently emits a light with a higher energy [68]. This emitted light can excite Photosensitizers involved in Photodynamic Therapy (PDT) which can trigger apoptosis based on the generation of Reactive Oxygen Species (ROS). Excitation of UC nanoparticles with a light of higher wavelength (808 nm) enables higher tissue penetration depth and improved therapeutics. Moreover the lifetime and radius of action of generated is in micron ranges which enables localized and tunable delivery. They can also function as a NIR contrast agents which provides a theranostic facet [69]. One of the interesting properties of Gold nanoparticles is that they can absorb a light at NIR region and liberate heat. These photo induced thermo responsive drug delivery systems have been promising cargo transport vehicle with a theragnostic features. Xiao et al synthesized Gold nanorods (GNR) based drug delivery system. They have capped the surface of the GNR with a single stranded DNA and their complimentary strand rich with CG basepairs and PEG. Presence of higher number of CG basepairs enables loading of Doxorubicin. As the GNRs were incited with NIR light, heat was generated which can break the hydrogen bonds between the host strand and the complimentary strand which enables the release of the drug [70]. Moreover GNR can also function as a contrast agent for CT, X-ray, two photon imaging and can also be used for photo thermal therapy [71].

Magnetic

Magnetic nanoparticles have been very attractive and extensively used particularly in the field of biomedicine. Their controlled sizes of few nanometers to tens of

nanometers place them at dimensions comparable or smaller than cells, viruses, proteins or genes. These magnetic nanoparticles obey coulomb's law which proves that one can manipulate these particles by external magnetic field. Due to their magnetic field enabled manipulations, magnetic nanoparticles facilitate intrinsic penetration into human tissues. This proves them to be potential candidates in a myriad of applications involving transport and/or localization of these nanoparticles or biological molecules (Magnetofection). Moreover their superparamagnetic behavior- when the size is brought down to few nanometers (<10nm) - aids them to function as a negative MRI contrast agent and also as a Hyperthermic agent [72]. Recently scientists used magnetic field mediated gated drug delivery of doxorubicin. Thomas et al. have synthesized magnetic core silica nanoparticles where the core was zinc doped iron-oxide magnetic nanocrystals and the surface was capped with *N*-(6-*N*-aminohexyl) aminomethyltriethoxysilane. Later the drug Doxorubicin was loaded into the carrier and the containment was capped with cucurbituril, which electrostatically bound to *N*-(6-*N*-aminohexyl)aminomethyltriethoxysilane. Upon application of alternating magnetic field (AMF), the containment released the drug due to the breakage of electrostatic interactions between cucurbit [6] uril and *N*-(6-*N*-aminohexyl)aminomethyltriethoxysilane. This nanovalve based drug delivery systems manifests the potency of the system [73]. Another important application of Magnetic field guided drug delivery was emphasized by Hernández et al where they have constructed DNA/ Mesoporous silica nanoparticles (MSN)/ SPIONs nanocarrier system for the gated drug delivery. They have loaded Mesoporous Silica nanoparticles with Doxorubicin and the surface was tagged with a single stranded DNA. The complimentary strand attached to SPIONs was basepaired with single stranded DNA on MSN to safeguard the freight. Upon exposure to AMF, the SPIONs tagged DNA liberated heat which breaks the hydrogen bonding between the nucleobases abetting in drug release. Due to their multitudinal modalities like imaging, therapy and transfection, Magnetic nanoparticles and their associated therapies have proved to be more important [74].

Ultrasound

Ultrasound triggered drug delivery systems have proved to be efficient as they can control spatiotemporal release of the drug to desired targeted sites despite the penetration depth. Moreover absence of harsh ionizing radiations or their non-invasiveness makes these systems more appealing. The mechanism of release of the drug is based on the generation of cavitation bubbles. As the sound waves are made of compressions and rarefactions, the liquid surrounding the bubble tries to move inside but the bubble tries to resist the compression. During the rarefaction, the size of the bubble increases. During the next cycle there is a compression where the liquid tries to push the bubble inside followed by rarefaction. Due to the repeated wave cycle, the bubble at certain point of time cannot withstand the inward pressure and an implosion happens which release the same amount of energy as increased temperature and pressure. Thus the energy required to cause the implosion of the bubble can be termed as threshold cavitation. The

cavitation threshold could be easily achieved if the lower ultrasound frequencies are used as lower frequencies give enough time for compression and expansion of the bubble compared to higher frequency [75]. Recent research has showed the use of perfluorocarbon based nanoemulsions as an efficient ultrasound triggered drug delivery systems. Formation of microbubble is achieved due to the acoustic droplet vaporization which is then subjected to ultrasound waves for cavitation. These micro bubble based nanocarriers systems have proved to be efficient drug delivery system [76]. Another diversified application of thermo liposomes is observed in theranostic field. Thermoresponsive liposomes loaded with both DOx and Ammonium Bicarbonate (ABC) have been used for both therapy and diagnostics. When the temperature was raised to 40°C, cargos in the liposomes were released. ABC at a temperature of 40°C, has the ability to dissociate into Ammonia, Water and Carbon-di-oxide (CO₂ bubbles [77]. These bubbles generated could be used as an Ultrasound imaging contrast agents. Moreover, Sonoporation, Ultrasound assisted transfection of the drugs has also been an additional advantage of these systems [78]. Though they are efficient, some of the disadvantages like sonolysis, disruption of the cells due to the increased temperature and pressure could be restricting their usage in clinical trials.

pH

The pH responsive nanosystems are still extant and much investigated in the current research world. Generally pH responsive systems should respond to the change the microenvironment of the system and release the cargo. These systems have created an impact particularly in cancer therapies as the local pH of the cancer cells is dissimilar from the normal cells which provided these systems an advantage. A deluge of nanocarrier systems have been constructed using a variety of polymers, copolymers, Liposomes, and pH activated bonds that can instigate the drug release. Recently fusogenic pH responsive liposomes have shown an enhanced therapeutic efficiency compared to normal liposomes [79]. Presently they have been integrated with hybrid nanosystems which can respond to a variety of stimuli (both Internal and external). Clawson et al displayed their adroitness in using pH for drug delivery applications. Though PEG has showed to improve the systemic circulation time, they have showed to display pessimistic effects after reaching the target site. They have showed to prevent the carrier entry into the cell or escape the carrier from endocytosis. These problems were subjugated by the pH responsive bond breaking system after reaching the target site which enables their entry and facilitates toescape the endocytosis. They have synthesized a lipid- polymer smart nanocarrier system based on the lipid-succinate- methoxy PEG (mPEG) conjugate which is hypersensitive to acid hydrolysis of diester succinate linker between PEG and lipid. Thus after reaching the niche of the cancer cells, PEG can be shed from the system enabling enhanced therapeutic efficiency of the system. Thus these nancarrier systems proved to be very efficient in cancer treatment [80].

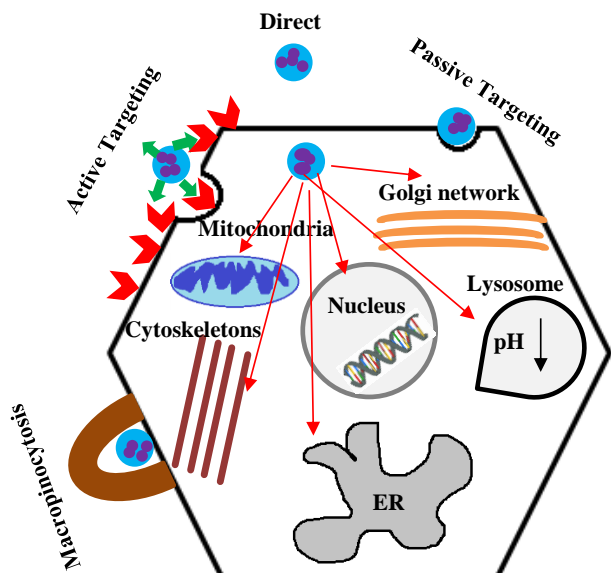


Fig. 4. Hexagon indicate cancer cell and the pathway of intracellular transport of nanoparticles. After internalization via one or more of the endocytic pathways i.e. passive targeting and active targeting. Then the drug released to the cell and interact with chromosome, mitochondria, Cytoskeletons, Golgi network, Lysosome and Endoplasmic reticulum (ER) system and cause apoptosis and arrest cell cycle.

Redox

Glutathione (GSH) mediated breaking of disulfide bonds have prone to be successful as Redox sensitive drug delivery systems. The mechanism of GSH triggered release is as follows: Sun et al. have constructed amphiphilic polyamide amine-*g*-polyethylene micelle where the entire main chain was filled with disulfide linkages. They have formulated a micelle loaded with Doxorubicin. These micelles were stable at normal physiological conditions but when counteracted with GSH, there was a reduction assisted disassembly of the micelle allowing releasing the drugs from the system. Though these systems proved to be effective they were not able to control the release of the drug and may be nonspecific [81].

Enzymatic

Altered expression on certain enzymes (eg: Protease, glycosidases) in certain tissues or cancers have been exploited to achieve enzyme triggered release. Some of the examples of enzyme triggered drug release have been discussed here. Generally enzyme modulated drug delivery is mediated by the enzymes in extracellular environs. Zhu et al reported Matrix metalloproteinases (MMP) mediated segmentation of short peptide sequence. They have modified the surface of the liposomes with TAT peptide and also with the PEG and monoclonal antibody attached short peptide sequence that can be cleaved by MMPs. Upon reaching the target via antibody mediated targeting, these PEGylated sequence were sliced and TAT protein empowered the entry of drug inside the cells. Though some of the examples are evident, much work needs to be established to explain the level of expression of these enzymes at the target sites and also needs to correlate the enzyme activity and in vivo drug release [82].

Analyte specific

Nanocarrier systems that are able to respond to certain analytes or change in their concentrations can attribute to Analyte specific drug delivery systems. These self-regulated systems plays a pivotal role in case of non-invasive delivery of insulin which should modulate the release based on the presence of local glucose concentrations in blood. Most investigated self-regulatory drug delivery system (DDS) includes phenylboronic acid and their derivatives based DDS. Kim et al synthesized polyboroxole block copolymers for insulin delivery. The hydrophobic neutral boroxole derivative turns charged as they encounter glucose or fructose which resulted in rupture of the micelle and release of the insulin. In spite of proof of concept of these systems much clinical research work need to be established to use them as a potential DDS [83].

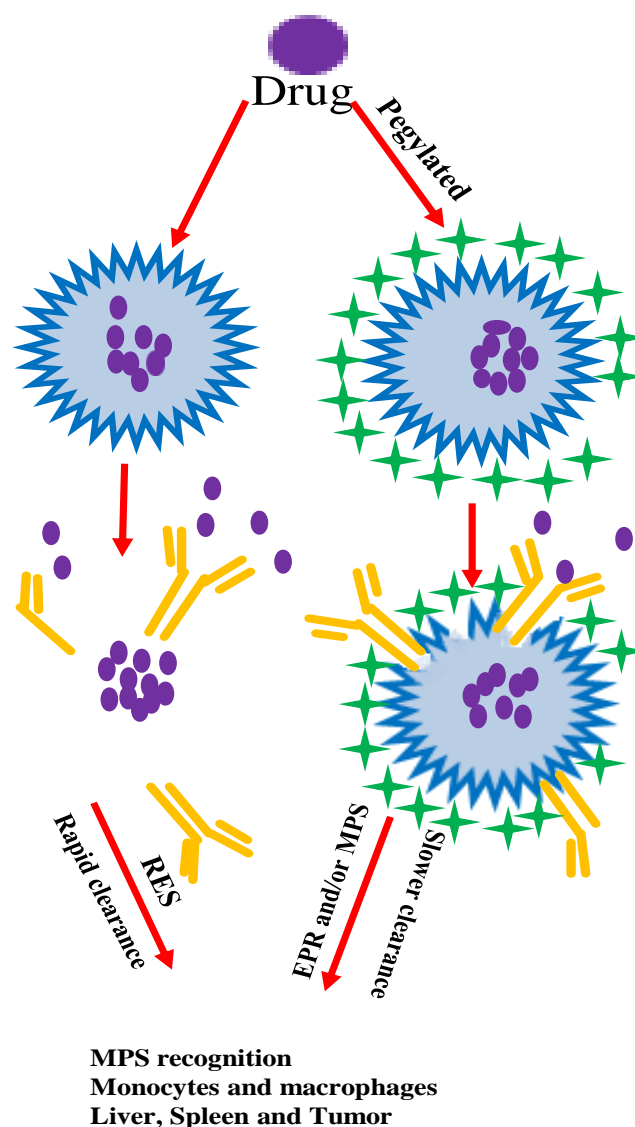


Fig. 5. Summary of the clearance of nanoparticles by the MPS recognition. PEGylated and non-PEGylated liposomal agents, could affect clearance system but the pharmacokinetics (PK) of nanoparticle carrier is affected by age, gender, the dose and regimen body composition, type of cancer and other drugs which is used for treatment. Purple circle show as Drug. Green stars show as polymer coating. Dark blue spike show as lipid membrane. Yellow Y as a receptor for opsonins.

Table 2. Stimuli responsive systems: Overview of dual/multiple-stimuli responsive nano-carriers for drug release.

Stimuli	Nanoparticle System	Drug/Cargo	Ref.
pH/Temp.	P(NIPAM-co-DMAAm-co-UA) NPs	DOx	[85]
	P(NIPAM-co-AA)-b-PCL NPs	PTX	[86]
	PLA-g-P(NIPAM-co-MAA) NPs	5-FU	[87]
	P(NIPAM-co-DMAAm)-b-PCL/PLA micelles	ADR	[88]
	mPEG-g-P(AA-co-MEA)-g-PNIPAM nanogels	DOx	[89]
	PNIPAM/PAA IPN hollow nanogels	INH	[90]
	P(NIPAM-co-Chitosan)-g-(MAA-co-MMA) nanogels	Caffeine	[91]
	mPEG-b-P(HPMA-Lac-co-His), mPEG-b-PLA and cy5.5-PEG-PLA mixed micelles	DOx	[92]
pH/Magnetic	Fe ₃ O ₄ NPs	DOx	[93]
	mPEG-b-PMAA-b-PGMA-Fe ₃ O ₄ NPs	ADR	[94]
	Fe ₃ O ₄ -capped MSNs	Dexamethasone	[95]
	MCM-TAA-Fe ₃ O ₄ -capped MSNs	DOx	[96]
	Fe ₃ O ₄ -coated PEG-b-PDEAEMA-b-PGMA	Chlorambucil & indomethacin	[97]
	Peptide mimic shell -magnetic nanocarriers (PMNCs)	DOx-Hydrochloride	[98]
	Fe ₃ O ₄ @SiO ₂ nanoparticles coated with PEG-poly(imidazole-L-aspartamide)	DOx	[99]
pH/Redox	PMAA-based nanohydrogels	DOx	[100]
	Poly(PDSM)-b-poly(HPMA)	DOx	[101]
	PCL-b-P(OEGMA-co-MAEBA)	CPT & DOx	[102]
	CS-SH and DS based LbL nanocapsules	BSA	[103]
	PEG-SS-PTMBPEC	DOx	[104]
	PEG-anti-bcl-2 oligonucleotide nanostructure	DOx	[105]
	PEG-SS-PDEA polymersomes	FITC-BSA/CC	[106]
Temp/Magnetic	Pluronic with Fe ₃ O ₄ nanoparticles	DOx	[107]
	P(OEGA-co-DMDEA) nanohydrogels containing orthoester bond and BADS	DOx, PTX	[108]
pH/Temp/Redox	PNIPAM-SS-P(THP-protected HEMA) micelles	Nile Red	[109]
pH/Redox/Magnetic	Fe(II) loaded PMAA microcontainers crosslinked by N,N-methylene-bisacrylamide and N,N-bis(acryloyl)-cystamine	DNR	[110]
Temp/pH/Guest molecule	DBHC micelle complexations of cucurbit[8]uril (CB[8]), PNIPAM and PDMAEMA	DOx	[111]

Hybrid system

Nanocarrier systems that can respond to two or more stimuli to enhance the therapeutic efficiency and incorporate theragnostic aspects could be termed as hybrid nanocarrier systems. This has been a highly investigated field of research in the current ages. Multitudes of hybrid carrier systems have been strategized to investigate its theranostic modalities. Recently, Patra et al. have strategized MRI-visual order-disorder micellar nanostructures for smart cancer theranostics. They have loaded Doxorubicin into a carrier with a magnetic core and a pH sensitive shell. Presence of both imaging and therapy makes these systems distinctive and imperative. Moreover the possibility of having both tissue and molecular imaging adds on to its importance. In spite of their advantages of

being multiple stimuli triggered release, much established work needed to use them for clinical trials [10].

Currently the usage of stimuli sensitive nanocarrier systems is in budding stage. As discussed earlier thermo responsive liposomes related DDS (thermo DOx) have proved to be successful and are currently in Phase II and Phase III trials. Compared to endogenous stimuli, exogenous stimuli have proved to be successful as they could be easily controlled and can be patient specific compared to the endogenous burst release phenomena [85]. Thanks to the advancement in materials science and biomedicine that has led to explore a myriad of stimuli responsive nano-carrier formulations and their efficacy. Though at this point of time it is difficult to express which stimuli responsive system would be efficient and productive, it is always better to have a simple and efficient nanocarrier that can be envisaged as a better drug delivery system.

Conclusion and future perspectives

Theranostics, associates with combining both therapeutic and diagnostic capability into one single moiety, a new protocol is anticipated to tailor a treatment based on the test results, thereby taking imaging and targeted drug delivery to new levels, providing more specific and efficient systems for the curing of disease. Emerging nanotheranostics is offering great opportunities to design and generate such hybrid agents capable of detecting and treating diseases in one single procedure. Such nano-technological applications are providing excellent opportunities to design and combine modeling imaging agents that could be functionalized to seek out specific diseased conditions and could be monitored with conventional clinical imaging modalities, wherein the detection modality is extensively allowed to run not only before or after but also through the treatment regimen.

Nanotheranostics will have to be developed in a much broader sense so that therapy and diagnostics can work hand in hand in successful realization of theragnostic agents that relies on the innumerable inherent properties and applications of use of Nanoparticles (NPs) for creating Targeted drug delivery systems with the possibility to localize them in specific sites of diseases and mitigate undesired side effects. The goal is to develop specific and individualized therapeutic strategies towards personalized medicine (PM), in light of the fact that the concurrent delivery and readout of efficacy or localization can be adapted to tailor treatment regimens for specific group of patients/individuals specific biomolecules. In the upcoming future, PM is expected to be the main focus of biomedical research with a view of advanced development in biomarker discovery, diagnostics, drug-delivery systems and biologics, PM will only be further strengthened. Therapy will be precisely chosen based on the heterogeneity of the cancerous tumor and the biomarkers present as more options for therapy are made available. As multiple Biomarkers develop on tumor cells, a single biomarker cannot be the only indicator addressing the cancer subtype. The interplay of specific biomarkers can give more information of the disease state and, furthermore, the treatment response. Correlation among various biomarkers and the disease class will require expansion into

more state-of-the-art molecular profiling techniques. Such biomarker discoveries will drive the design of diagnostic systems towards multiplexing, and furthermore into efficient treatment monitoring.

In general, Cancer drug delivery systems will move towards more specific Biomarker targeting making systems more stable and eventually increasing the blood circulation time of the drug with improved cellular uptake reducing the possibilities of Multi drug resistance (MDR) and toxic side effects that could otherwise hamper the effective and efficient recovery. Nanoparticulate clearance after

successful theranostics will also be the main key of biomedical studies in the upcoming future.

The introduction of nanotheranostics into routine health care has still a long way to go, since evaluations on cytotoxicity, genotoxicity, and immunotoxicity of prospective nanotheranostics, demonstration of cost-effectiveness, and availability of appropriate accessible testing systems are still required. Despite notable progress, there remain no/only a few nanotheranostic agent/particle systems that are sufficiently sophisticated to meet clinical standards.

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