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Review Article

Nano-Theranostics – Innovative Synergy of Therapeutics, Diagnostics, Prognosis and Continuous Monitoring Using Multifunctional Nanomaterials

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Abstract

Theranostics is a new promising medical paradigm that synergistically utilise therapeutic and diagnostic capabilities of agents for diagnosis, drug delivery or therapy and therapy response recording. This field is further evolving fast into the nanotheranostic field which is immensely benefitted by multifunctional nanomaterials that can enhance not only the imaging quality but also the specific targeting of disease sites. Nanotheranostic agents will help to reduce toxicity and the time of both diagnosis and therapy and help to develop personalised therapies.

The present review article explores recent advances of this fascinating field and its future.

Introduction

Theranostics

Theranostic a term coined by John Funkhouser (PharmaNetics) in 2002 for developing diagnostic tests that are linked to the application of specific therapies [1,2]. Theranostics [1-3] is the combination of therapy and diagnostics performed using single agent. Alternatively, theranostics can be defined as the diagnostic agent that can be used for therapeutic purpose so that we do not need two or more separate agents or technologies for diagnostic, prognosis and therapy or treatment. The "theranostics" field is emerged to develop more specific, individualized therapies for various diseases by combining diagnostic and therapeutic capabilities into a single multifunctional agent. This new medical paradigm is evolved from an urgent need of rapid diagnosis and treatment of life-threatening diseases such as cancer. In addition, theranostics treatment methodology will help the development of personal medical treatments that are more specific to consumer's needs to address current drawbacks such as improved prognosis and reduce dose-dependent toxicity or side-effects.

A theranostic system may be comprised three parts as follows.

- A targeting theranostic agent: Directs itself to a molecular target on the surface of a cell or tumor.
- A(chelating) imaging agent: That can locate the specific target or a therapeutic drug and help delivery of treatment to the specific target site.
- A linker: Connect the two entities such as imaging agent and therapeutic agent.

The integration of multiple moieties into a single agent for imaging and therapeutic purposes provides a powerful new paradigm for advancing treatments against cancers and other diseases.

Radioiodine is one of the first theranostic agent used in the 1940s to image and treat thyroid cancers [4]. The first molecular imaging using radioiodine was performed by Dr. Benedict Cassen in 1950 at UCLA [5,6]. He successfully imaged the gland using the rectilinear scanner and revealed biologic characteristics of the thyroid tissues using radioiodine. An example of radioiodine theranostic conducted by Dr. Cassen is provided in Figure 1. Figure 1 depicts two patients A and B who were diagnosed with advanced ovarian cancer. As seen from Figure 1, the pre-treatment F-18 FDG PET imaging was successfully used to visualize multiple cancerous lesions in the neck and abdominal cavity of both patients. However, the imaging was not able to evaluate the therapeutic response of the subsequent chemotherapy. Patient A seemed to have achieved complete remission after the chemotherapy whereas patient B progressed to disease status after the chemotherapy.

Nano-Theranostics

Nanoparticles (NPs) offer enhanced physico-bio-chem and optoelectronic properties due to high specific surface area, shape and (quantum) size effects and can be used to enhance absorption of radiations that can deliver concentrated dose of radiation locally to the disease site without affecting healthy cells [7-12]. These improved properties of NPs have been used to achieve fast and improved continuous imaging or diagnosis (during the treatment) and site-selective drug delivery individually. Due to their nanometer dimensions, NPs can easily overcome cellular, anatomical, and physiological barriers including the blood-brain barrier and detect changes at cellular and molecular levels. Multifunctional nanomaterials can play complementary roles

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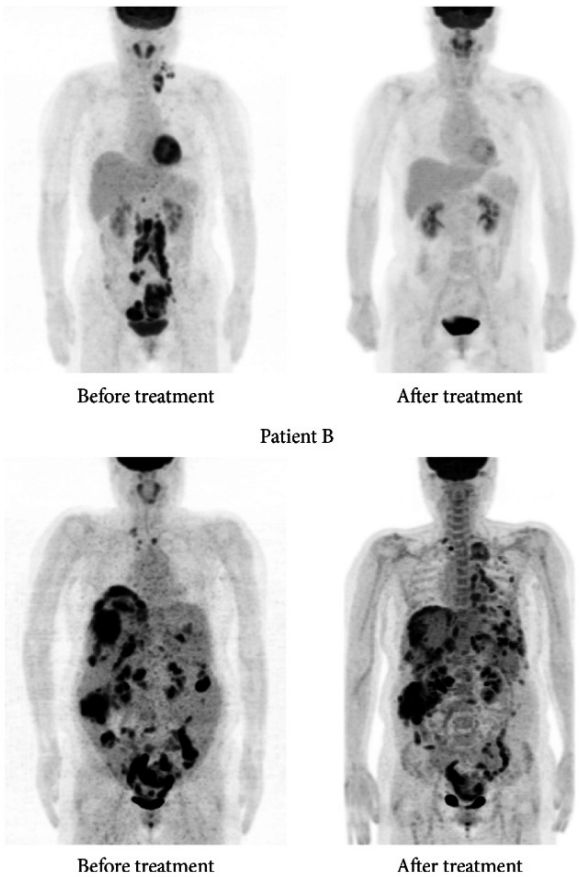

Figure 1: Patients A and B were diagnosed with advanced ovarian cancer. Pretreatment F-18 FDG PET imaging successfully visualized multiple cancerous lesions in the neck and abdominal cavity. However, the imaging was not able to predict the therapeutic response to the subsequent chemotherapy. Patient A achieved complete remission after chemotherapy; however, patient B progressed to disease status after chemotherapy. Source: Biomed. Res. Int. 2016; 2016: 1680464. Copyright © 2016 Byeong-Cheol Ahn.

in molecular detection and detect changes more efficiently and will redefine diagnostic and therapeutic paradigms in the near future. In addition, the modern therapeutics such as proteins or peptides [13], antibodies [14,15], and water-insoluble drugs [16] require specialized delivery systems to maximize therapeutic efficacy. Furthermore, various nanoscale delivery systems are being developed to achieve precision in targeted drug therapy and to improve drug bioavailability and reduce cytotoxicity.

The same NPs should able to load the drug and co-deliver the drug to the specific site for the treatment and perform continuous imaging of the site during the treatment task also, and not just before and after the treatment. Therefore, the nanotheranostic agents (NTAs) should exhibit both multifunctional and multi-tasking properties.

Challenges & Characteristics of Developing NTAs

However, there are certain challenges that need to be addressed during the development of NTAs. One of the key challenges is that both diagnostic and therapy need sufficient accumulation of NTAs at the specific disease site. Other challenges included are stability of NTAs (long half-lives in blood circulation), broad surface chemical functionality to adsorb and desorb drug molecules, maximum loading of the drug per particle, toxicity, avoiding innate immuno system, biodistribution and clearance, etc. [17].

The nature of disease and disease site area need to be considered while developing NTA targeting strategies to achieve maximum efficacy. For example, for the cancer treatment, NTAs should exhibit permeability-and-retention (EPR) effect for tumor targeting. Therefore, NTAs need a suitable biomarker that can selectively expressed onto the cancer cell surface and cognate a binding vector for its maximum loading on the particle for efficient imaging and/or therapeutic efficacy. These strategies may help NTAs to have maximum EPR with long circulation half-lives and avoid the innate immuno system recognition.

Pharmacogenetics and NTAs

The discovery of genetic, genomic and clinical biomarkers have provided opportunities for the personalised medicine treatment to accurately predict a person's susceptibility/progression of disease, the patient's response to therapy, and ways to maximize the therapeutic outcome specifically to a particular patient. Thus, it is possible obey "4R" of medical services by providing the right treatment, to the right patient, at the right time, and at the right cost [18]. The personalised medicine emphasizes on genetic make up of individuals that can becorrelated with difference in drug therapy. Genetic variations in humans are recognized as an important determinant of drug response variability because different patients respond differently to the same drug due to 20–95% variability in genetics. As pharmacogenomics studies help to understand the effect of human genetic variations on the patient's response to drugs by considering the drug's pharmacokinetic properties such as absorption, distribution and metabolism. Aslo these studies help to reduce or avoid the adverse drug reactions and optimizing the drug dose by identifying drug responders and non-responders. The U.S. Food and Drug Administration (FDA) considers pharmacogenomic principles for making safer and more effective drugs. Nanoparticles may help to develop both biomarker identification and selective/target drug delivery, and resultant NTAs can be used for the personalized treatment of diseases such as cancer.

Biochemical Conjugation Reactions to Develop NTAs

NPs have been used in biomedicines, drug and gene delivery, imaging, sensing and diagnostics. However, the surface of NPs needs to be modified to enhance biocompatibility and functionality for the *in vitro* and *in vivo* applications, particularly in delivering locally and recognizing biomolecules.

NPs based biosensors enables the detection of biomolecules under extremely low concentration by using optical [19], and electrical or electrochemical signal [20]. Size-dependent physical and chemical properties of NPs are used for high throughput labelling and detection of bioanalytes [21,22]. A number of nanosensors are used for the detection of proteins, nucleic acid, bioactive molecules, bacterial and viral agents [23,24]. NPs with unique optical property can be directly labelled with a biomolecules such as nucleic acid

probe, antibody, enzyme, aptamer or small bioactive ligands for the detection purposes [25].

A typical functionalised NTA is depicted in Figure 2. As depicted in Figure 2, biomolecules such as RNA and DNA are generally attached on the internal or external surfaces of a suitable nanocarrier (e.g. Au nanoparticles) through physical adsorption and/or chemical bonding or biofunctionalisation [26]. Polyethylene glycol (PEG) also attached with nano-carriers to improve the solubilisation of NPs in aqueous solutions. Conde *et al.*[26] have reviewed the past 30 years of research on biofunctionalisation and surface chemistry of inorganic nanoparticles for nanomedicine applications. Additionally, Werengowska-Ciećwierz *et al.* have reviewed the most important ligand-nano carrier and drug-nano carrier bioconjugations [27]. Some of the covalent bonds between targeted ligands and nano-carriers are amide, thioether, disulfide, acetyl-hydrazone and polycyclic. Some of the most important nano-carriers are liposomes, micelles, polymeric nanoparticles, dendrimers, carbon nanotubes, and nanohorns that could be effectively applied in targeted anticancer therapy.

In the targeted anticancer therapy constructs are form by connecting the ligands to the carriers of chemotherapeutic agent. The resultant constructed structure facilitates the bioconjugation with suitable receptors of cancer cells which supports the over expression of cancer cell receptors and the affinity of ligand to receptor [28-30]. The chemotherapeutic agent is thus delivered to the most resistant cancer cells with internal long time circulation there by guarantying the high drug concentration *insitu* the tumor. Also, the drug cannot be released back to the blood circulation. However, immunogenicity of the ligand is important from the safety and efficacy point of view [31]. Hydrophilic and hydrophobic drugs, proteins, vaccines, and biological macromolecules can be deliver using nanoparticles as carriers. The three main types of gene delivery systems have been used for the delivery of antigens for vaccination are: viral vectors, nonviral vectors (in the form of particles such as nanoparticles, liposomes, or dendrimers), and the direct injection of genetic materials into tissues using so-called "gene guns" [32]. Nanoscale drug delivery mechanisms can affect drug release properties and intracellular entry capability and may help minimise side effects. It may be useful to directly treat the root-cause of diseases instead of the symptoms of the illness. Generally nanoparticles are used for site-specific drug targeting for treating various diseases including cancer, human immuno deficiency virus infection, and central nervous system disorders, and better than microparticles found to be better than microparticles[33,34]. Higher surface to volume ratio of nanoparticles are helpful to reduce the dose and frequency of administration and increase the patient compliance. Some of the NTAs are discussed as follows.

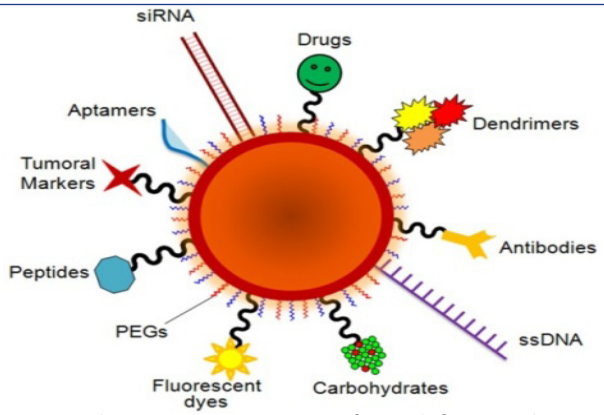

Figure 2: Schematic representation of a multifunctional nanocarrier. These innovative NPs comprise of nucleic acids such as RNA and DNA used for gene silencing approaches and in colorimetric assays, respectively. Aptamers and anticancer drug molecules are also used for delivery to the target tissue. Carbohydrates may be useful as sensitive colorimetric probes. PEG is used to improve solubility and decrease immunogenicity. Responsive nanocarriers can also trigger reaction upon external stimuli through the functionality of valuable tumor markers, peptides, carbohydrates, polymers and antibodies that can be used to improve nanocarrier circulation, effectiveness, and selectivity. Multifunctional systems can also carry fluorescent dyes that are used as reporter molecules tethered to the particle surface and employed as tracking and/or contrast agents. (Source: Conde J, Dias JT, Grazú V, Moros M, Baptista PV *et al.* (2014) Revisiting 30 years of biofunctionalization and surface chemistry of inorganic nanoparticles for nanomedicine. Front Chem. 2: 48. Copyright: Frontier in Chemistry.)

Iron Oxide NTAs

Magnetite or hematite based Iron oxide nanoparticles (IONPs) exhibit saturation magnetization (Ms) values at room temperature, particularly those made from pyrolysis which resulted in good crystallinity. IONPs which are less than 20nm exhibit super paramagnetic properties. The thermal energy is sufficient to overcome the anisotropy energy of each magnetic nanoparticles which leads to random fluctuation of the magnetizations that macroscopically result in zero net coercivity and magnetic moment. Due to the superior magnetic properties, inherent biocompatibility and inexpensiveness, IONPs are used as contrast probes for magnetic resonance imaging (MRI). The high magnetic moments of IONPs can reduce T2 relaxation time and attenuate a signal on a T2 or T2* weighted map. This signal alteration can be used to target specificity to report abnormal biological activity. During synthesis of IONPs, additives or ligands such as hydrophilic polymers (polyvinylpyrrolidone (PVP), dendrimer, polyaniline and dextran or its derivatives) are used to passivate the nanocrystal surface to avoid the aggregation of nanoparticles. Sawant *et al.*[10] has demonstrated the use of nanosize Tc^{+99} Fe_2O_3 for the lung imaging by lung photoscientigraphy. Figure 3 Depicts a typical lung image of a healthy volunteer obtained using nanosize Tc^{+99} Fe_2O_3. This method can also be used to treat lung disorders particularly tumors while imaging the same.

Several dextran-IONP have used as MRI contrast agents in clinical trials. Feridex particles comprised of superparamagnetic dextran-IONP [35] developed by AMAG Pharmaceuticals are FDA approved for the detection of liver and spleen lesions.

Kohler *et al.*[36,37] have coupled an anti-cancer drug methotrexate (MTX) onto an aminated IONP surface and demonstrated that the particles can be internalizing into cells and accumulated in lysosomes where the drug molecules were released due to the

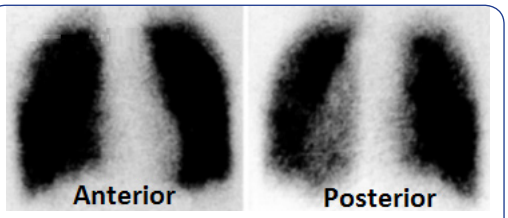

Figure 3: Photoscintigraphic sections of the human lung imaged using iron oxide NTAs.

low pH and the presence of proteases. Thus, MTX-immobilized poly (ethylene glycol) magnetic nanoparticles can be used as agents for MR imaging and drug delivery to treat cancer. Hwu *et al.*[38] have coupled paclitaxel (PTX) to IONP surfaces through a phosphodiester moiety at the (C-2')-OH position and around 83 PTX molecules coupled per nanoparticles. The release of PTX is found to be more effective when exposed to phospho diesterase. Huh *et al.*[39] and Lee *et al.*[40] have used *meso*-2,3-dimercaptosuccinic acid (DMSA) to modify IONPs, and used Succinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate (SMCC), a heterobifunctional protein crosslinker to couple herceptin antibody molecules onto the particle surface. Herceptin can be used as both targeting agent and therapeutic agent to detect cancer using the magnetic resonance method and treat cancer cells also. Instead of using the covalent coupling method, drug molecules can be co-capsulated with IONPs into polymeric matrices. Jain *et al.* have encapsulated doxorubicin (DOX) and PTX, along with oleic acid coated IONPs in pluronic-stabilized nanoparticles [41]. Yu *et al.* also loaded DOX into anti-biofouling polymer coated IONPs [42]. These DOX loaded nanoconjugates have exhibited better pharmacokinetics and therapeutic effects than DOX alone in a Lewis lung carcinoma xeno graft model due to the anti-biofouling feature of the particles. To compare the antiproliferative effect of drugs (paclitaxel, doxorubicin and their combination) in solution and loaded in magnetic nanoparticles (MNPs), MCF-7 cells were treated with the drug either in solution or loaded in MNPs and cell viability was measured using an MTS assay on day 5. Small active molecules can also be loaded into porous nanostructures through physical absorption. Spindle-shaped β-FeOOH NPs made from $FeCl_3$ hydrolysis, and used a three step, called "wrap-bake-peel" treatment to create hollow IONPs. DOX can be loaded into such hollow nanoparticles via simple physical absorption and then released from the nanostructures in a sustained manner under physiologic conditions [43-45].

Cheng *et al.* [46] have developed porous IONPs with a nano-size cavity (~2-4 nm) using controlled oxidation and acid etching of Fe particles and cisplatin is diffused into these nano cavities and subsequently coupled herceptin onto the particle surfaces to confer targeting specificity. The resulting conjugates have exhibited selective affinity to ErbB2/Neu-positive breast cancer cells with IC(50) reaching 2.9 muM, much lower than 6.8 muM needed for free cisplatin. A sustained cytoxicity is attributable to the controlled release of cisplatin from IONPs. Thus, the low pH-responsive Fe_3O_4NTAs can be used as a cisplatin delivery vehicle for target-specific therapeutic applications. There are several other examples of NTAs used for gene and cancer therapies [47,48]. Seven patients with metastatic breast cancer were infused with epirubicin-loaded IONPs (100 nm in diameter, at 0.5% of the estimated blood volume), and after that a magnetic field was established around the tumor. The magnetic field proved successful in directing the ferrofluid to the tumor to induce tumor regression.

Namiki *et al.* [49] have screened cationic lipid coated IONPs. Additionally, Authors [49] have developed lipid coated IONPs "LipoMag" and compared it with "PolyMag" and found that "LipoMag" out performed commercial "PolyMag" in both transfection and gene knock down in all 13 tested cell lines. The authors found one sequence, siRNA, after screening several siRNA with the maximum percentage knock down of EGFR mRNA and performed 2'-OMe modification on the uridine residues of the sense strand, and yielded a modified sequence with similar knock down effect but reduced cytokine induction compared with the parent sequence. Subsequently, NPs were loaded with modified siRNA and evaluated their therapeutic potency in two gastric cancer models which resulted in a 50% reduction in tumor volume after a 28-day treatment, and the inhibition of both angiogenesis and the induction of apoptosis. The gene knockdown is found to be significant only during application of magnetic fields at the tumor sites. IONPs are also used as a NTAs in hyperthermia because IONPs can act as antennae in an external alternating magnetic field (AMF) to convert electromagnetic energy into heat. Phospholipid coated IONPs were injected into a subcutaneous tumor model in F344 rats, and were exposed to an AMF. The AMF in conjugation with IONPs raised the temperature of tumor above 43°C to cause tumor regression whereas no tumor regression was found in the control group treated without IONPs. Also, the Fab fragment of anti-human MN antigen-specific antibody is chemically anchored onto IONP surfaces and resultant INOPs are administrated systemically into tumor-bearing mice. IONP particles showed high tumor uptake, presumably due to an antibody-antigen interaction, and induced efficient tumor hyperthermia when exposed to an AMF [7].

Quantum Dot NTAs

Quantum dots (QDs) are light-emitting nanocrystals made from semiconductor materials. QDs possess unique size-dependent optical properties such as photo-stability and chemical stability and a narrow emission spectrum [9]. QDs of CdSe, CdTe and PbS, can emit light in the visible spectrum based on their particle sizes but are not efficient in *in vivo* applications due to the limited tissue penetration distances of visible light. Therefore, new QDs with near-infrared emission have been developed using CdTe/CdSe, Cd_3P_2, InAs/ZnSe and InAs/InP/ZnSe. Also an inorganic coatings such as ZnS on the QDs surface has proved useful in enhancing the photo luminscent quantum efficiencies of the resultant QD particles and also sulphur moity of the ZnS layer is use as a species-mounting site. Coatings also help to increase the biofate of QDs. For example, cysteine modified QDs are water soluble with a hydrodynamic size less than 5.5 nm have found to be rapidly excreted via renal

clearance, instead of traping in reticulo endothelial system (RES) organs, such as liver and spleen. In addition to ligand-exchange, ligand-addition-based surface modification techniques using a group of amphiphilic compounds, such as phospholipids, amphiphilic saccharides, acrylic acid polymers and others are used to improve the efficiency of theranostic QDs.

Smith *et al.* [50] have used a triblock copolymer (comprise of a polybutylacrylate segment, a polyethylacrylate segment, a polymethacrylic acid segment and a hydrophobic hydrocarbon side chain) to modify QDs and conjugated onto them a prostate-specific membrane antigen (PSMA) targeting antibody. These QD-nano-conjugates are administrated into prostate cancer bearing mice and found to be accumulated in the tumor area due to both the EPR effect and specific antibody-antigen interaction. Nurunnabi *et al.* [51] have developed water soluble QDs-Herceptin-PEG-10,12-pentacosadiynoic acid (PEG-PCDA), and stabilized by cross linking the coating shell using the UV irradiation. These nano theranostic QDs exhibited an efficient tumor targeting rate and impressive therapeutic effects on the MDA-MB-231 tumor model. Bagalkot *et al.* [52] have developed a QD-aptamer(Apt)-DOX conjugate [QD-Apt(Dox)], and used for simultaneous cancer imaging, therapy and therapy monitoring. The fluorescence activities from QD and DOX are attenuated by their interaction with DOX and RNA, respectively, and are both in a quenched state in the nanosystem. Subsequently, these resultant QDs are delivered into targeted tumor cells, where DOX is gradually released from the system leading to initiation of therapeutic functions as well as the recovery of QD fluorescence. Yuan *et al.* [53] have loaded MTX onto QD surfaces via reversible physical adsorption to induce photo luminescence quenching. The reversible physical adsorption of MTX is reversed when exposed to higher affinity species such as DNA and change in coating led to a restoration of the photo luminescence. This process can be potentially used to monitor the delivery of drugs. QDs are encapsulated in poly(maleic-anhydride-alt-1-decene), and further surface-modified with dimethylamino propylamine to impart positively charge. These QDs have out-performed polyethylenimine (PEI) by more efficient delivery and significantly reduced toxicity. Qi *et al.* [54] have converted some carboxyls to tertiary amines with N,N-dimethylethylenediamine on poly(maleic-anhydride-alt-1-decene) coated QDs resulting in having two functional groups on the particle surface. These QD particles have both steric and electrostatic interactions that are highly responsive to acidic endosome/lysosome organelles. As siRNA delivery vehicles, these QDs have showed a 10-20 fold increase in silencing effect and a 5-6 fold decrease in toxicity as compared to other common delivery agents, such as lipofectamiane, JetPEI and TransIT. In addition to loading siRNA via electrostatic force, covalently coupling of siRNA to QDs has been investigated. Derfus *et al.* [55] have covalently conjugated siRNA along with a tumor-homing peptide F3, onto QDs and studied the siRNA release. Also, QDs modified with amine functionalized polymer polydiallyldimethyl ammonium chloride (PDDAC) have been complexed with MMP-9-siRNA and utilized to modulate the activity of matrix metallopeptidase 9 (MMP-9), which is a main component of the blood brain barrier (BBB) in brain microvascular endothelial cells (BMVEC). An increase in the expression level of tissue inhibitor of metalloproteinase-1 (TIMP-1), a natural inhibitor of MMP-9 that functions to maintain the basement membrane integrity is found along with an increase in collagen I, IV, V expression and a decrease in endothelial permeability. QDs can be used as either photo sensitizers or carriers in photodynamic therapy because as a photo sensitizer, QDs can be activated by light and transfer the triplet state energy to nearby oxygen molecules to cause cell damage. Tsay *et al.* [56] have modified QDs using streptavidin and conjugated onto them biotinylated pDNA and found that the generation of reactive oxygen intermediates (ROI), through nitroblue tetrazolium (NBT) assay upon photoactivation of QDs. Photo activated ROI can impart elicit damage to purine and pyrimidine bases. However, the quantum yield of QD-generated singlet oxygen is typically less than 5%, which is much lower than that of classic photosensitizers (40–60%). Similarly, QDs conjugated with photosensitisers such as Ir-complex [57], phthalocyanine (Pc4) [58], mmeso-tetra(4-sulfonatophenyl) porphine dihydrochloride (TSPP) [59]. In the latter two cases, the QDs worked as drug carriers and are not directly involved in the photodynamic therapy. However, in the Samia *et al.*'s NTAs [58], the QD also acted as an energy hub, which transferred energy to phthalocyanine (Pc4) to activate its photodynamic therapy (PDT) function.

Gold NTAs

Gold nanoparticles (Au NPs) exhibit unique characteristics such as strong surface plasmon absorption, stability, biosafety, and ease of modification and therefore used for building up functional agents for both imaging and therapy applications. Because of its unique size-dependent opto-electronic properties, Au NPs have been used for imaging purposes in computed tomography (CT), photoacoustics and surface-enhanced Raman spectroscopy (SERS). Spherical Au NPs of 10nm exhibit characteristic surface plasmonic absorption at around 520nm which can be red-shifted to maximum of 575nm with a maximum of 99.4nm shift due to an increase in the Au particle size. Moreover, a change of the nanoparticle shape to rod-like can push the absorption to the NIR region (650-900 nm), therefore, Au NPs are used as probes in photoacoustic imaging or mediators in photothermal therapeutics. Due to the strong interaction between thiol and Au, the surface modification of Au nanostructures is conducted with a bifunctional compound where thiol terminal immobilized onto the Au NP surface while leaving carboxyl/amine terminal groups exposed for bioconjugation. On the other hand, biomolecules can be pre-thiolated and loaded onto particles. Thiolated DNA oligos have used for stabilizing Au NP colloids and the resulting conjugates have been investigated as gene therapy agents. Monodentate ligands are better than multidentate species to achieve higher loading capacity. Bhumkar *et al.* [60] have used chitosan as a reducing agent and coating material to make highly positively charged Au nanoparticles. These resulted chitosan-Au NPs are found to be highly efficient in loading insulin (53%) via electrostatic interaction and used as a diabetic model to control postprandial hyperglycemia. A drop in their blood glucose

level in diabetic rats was observed 2 hours after the administration of insulin loaded chitosan-Au NPs. Cheng *et al.* [61] found that a PDT agent, Pc4, can be directly adsorbed onto PEGylated Au NPs with high efficiency. The Au NPs can work well as a drug carrier and reduce the time of Pc4 delivery to less than 2 hours as compared to that of 2 days for free drug. Prabaharan *et al.* [62] utilized an amphiphilic-block-copolymer-coated Au NP formula for tumor targeting and drug delivery. A nanostructure consisted of a Au NP core, a hydrophobic Poly(aspartic acid (PAsp) inner shell and a hydrophilic, folate-conjugated PEG outer shell PEG-OH/FA is used to load 17 wt% DOX through covalent conjugation onto the hydrophobic inner shell. This nano system possesses both a tumor targeting mechanism (folate on the outer layer) and an intracellular drug release mechanism (hydrazone linkage of DOX on the inner layer). Thomas *et al.* [63] have used branched Polyethylenimine (PEI) to confer gene loading capacity to Au NPs and transfection potency was increased by 12 times compared to the branched PEI. In addition to electrostatic forces, therapeutic genes can also be loaded onto Au NPs through covalent linking. Moreover, thiolated antisense DNA oligos can be directly loaded onto Au NPs with high efficiency. Au NPs loaded with antisense DNA showed a high translocation rate and a prominent gene knock down efficiency in a cellular study with eGFP-expressing C166 cells. The unique surface plasmon resonance feature of Au NPs is used to serve as energy transducers in photothermal therapy. After AuNPs are concentrated in tumor areas, laser irradiation can convert light into heat and kill adjacent cancerous cells. This treatment is active only within the limited illumination area as compared to conventional drug delivery therefore it help to minimise normal tissue damage. However, spherical Au NPs with a characteristic absorption at 500-600 nm are not appropriate agents for such applications. Therefore, the configuration of AuNPs need to change to a nanorod, nanocage or nanoshell, to shift the absorption to the NIR region, to use in hyperthermia. PEG coated Au nanocages [64,65] are found to be accumulated in a U87MG xenograft model, and able to increase the tumor surface temperature to 54 °C within 2 min upon exposure to NIR light. In another study, Lu *et al.* [66] has used α-melanocyte-stimulating hormone (MSH) analog, [Nle^4, D-Phe^7] α-MSH (NDP-MSH), to couple onto Au nanoshells. The resultant nanoconjugates are administered to a B16/F10 melanoma model and found accumulated in the tumor in large quantities by NDP-MSH. Further, the efficient ablation of B16/F10 melanoma is found in the tumor that is exposed to the laser illumination only and not in the contralateral tumor which did not receive the illumination. This success of photothermal therapy is validated histologically and PET studies. [^{18}F] fluorodeoxyglucose (^{18}F-FDG) PET found a remarkable decrease in tumor uptake which indicated a drop in a metabolic activity upon the photothermal therapy. Lu *et al.* [67] also demonstrated the use of Au nano shells as light-controllable siRNA carriers. The particles are conferred with tumor targeting specificity by imparting folic acid and siRNA, with a sequence that targets NF-κB P65 to the nanoparticle surface via thiol-Au interaction. A stable thiol-Au association may help to carry the siRNA pay load on the nanoparticle surface even after cell uptake, and will get destroyed upon NIR light irradiation. The NIR light irradiation can damage the endolysosomal membrane to a release of siRNA into cytoplasms which is confirmed by the observation of light-inducible siRNA release and subsequent NF-κB P65 down regulation both *in vitro* and *in vivo* experiments. Moreover, the downregulation of NF-κB P65 is resulted in an increased sensitivity to chemotherapy which is evidenced by an improved therapeutic index when such photothermal therapy was combined with the irinote can treatment.

Carbon Nanotube NTAs

Carbon nanotubes (CNTs) exhibit a graphite-like structure which is inert and inhibitive to most of the conjugation chemistry but have potential applications in Raman and photoacoustic imaging and drug delivery [68, 69]. CNTs have found to be taken up by cells but the detailed mechanisms underlying such efficient cell penetration are still unclear. Also CNTs may effectively cross biological barriers and act as nano-vectors for the therapeutic agent delivery. The internalisation of CNTs by cells can occur via different routes such as endocytosis and passive diffusion depending upon surface coatings. The organic functionalisation of CNTs can improve their solubility and biocompatibility profile and their manipulation and integration into biological systems and use in the delivery of drugs, antigens and genes [70]. A high degree of CNT functionalization has found to reduce toxic effects [71]. Water-soluble *f*-CNTs and CNTs coated with proteins [72], polymers [73] and single-stranded DNA [74] can interact with mammalian cells, leading to their cytoplasmic translocation [75,76] whereas ammonium-functionalized cationic nanotubes condense and deliver plasmid DNA (pDNA) intracellularly, leading to enhanced marker gene expression [76,77].

Biological systems are highly transparent to 700nm to 1100nm near-infrared (NIR) light. The strong optical absorbance of single-walled carbon nanotubes (SWNTs) is an intrinsic property of SWNTs which can be used for optical stimulation of nanotubes inside living cells to afford multifunctional nanotube biological transporters [72,78]. Samori *et al.* have coupled MTX onto 1,3-dipolar cycloaddition functionalized CNTs [79]. These nanobio-hybrid conjugates are internalized into human cells including breast cancer cells and found that the cytotoxic activity is strongly dependent on the presence and type of linkers [80]. Phospholipid-CNT conjugates have been used for both imaging and therapy. siRNA is coupled to CNTs via a disulfide bond, and the resultant CNT transporter exhibited high transfection efficiency, outperforming lipofectamine in inducing RNAi. The selective cancer cell destruction can be achieved by functionalization of SWNT with a folate moiety, the selective internalization of SWNTs inside cells labelled with folate receptor tumor markers, and NIR-triggered cell death, without harming receptor-free normal cells. Therefore, the transporting capabilities of CNTs combined with suitable functionalization chemistry along with their intrinsic optical properties can be exploited for drug delivery and cancer therapy. Additionally, the coupling of either Pt(IV) prodrug or PTX onto PEGylated CNTs can improve the pharmacokinetics and therapeutic effects because PEGylation brings an extra stability to CNTs. PTX can be coupled through a cleavable ester bond to the

nanotube surface and the resultant construct is tested in a murine 4T1 breast cancer model. The nanoconjugates exhibited a 10-fold increase in tumor homing than PTX alone and prolonged the circulation half-life of the nanoformula. This formula has showed better tumor suppression outcome than clinically used taxol [81]. The strong optical absorbance of CNTs in the NIR region has made it a potential agent in the photothermal therapy. When irradiated by NIR light, internalized CNTs in cells are capable of triggering endosomal rupture and cell death. This is demonstrated by Moon *et al.* in a human epidermoid mouth carcinoma model that the combined treatments of PEGylated SWNT and NIR irradiation led to the eradication of tumors with no observation of recurrence over 6 months [82]. On the other hand, Ghosh *et al.* have encapsulated CNT using DNA which can lead to improved heat emission efficacy and found to induce complete tumor eradication after the internalisation of nano-conjugates in a PC3 xenograft model intratumorally and subsequent irradiation [83]. Despite potential applications of CNTs as agents a few key issues such as their non-biodegradability and chronic and longitudinal damage to the hostdue to residual CNTs and a lack of a standardised protocol to prepare high purity CNTs at small or large scale are restricting their clinical translation.

Silica NTAs

Silica is generally considered as a safe and has been used in surgical implants. Silica nanoparticles themselves do not have characteristics for imaging but are an excellent platform that allows facile loading of a broad range of imaging and therapeutic functions, thus making them potential NTAs. It is easy to create multiple chemical functionality of the surface of silica nanoparticles and encapsulate small molecules, IONPs and QDs or these nanoparticles can also be easily incorporated into silica matrices to combine both magnetic and optical properties [84,85]. Roy *et al.* [86] have used ultrafine organically modified silica-based nanoparticles (diameter ~30 nm) to trap water-insoluble photosensitizing anticancer drug 2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide, using the micellisation method. The resulting drug-doped nanoparticles are found to be an spherical, highly monodispersed, and stable in an aqueous system and the entrapped drug is more fluorescent in an aqueous medium than the free drug, permitting the use of fluorescence bioimaging studies. Irradiation of the photo sensitizing drug entrapped in nanoparticles results in efficient generation of singlet oxygen, due to the inherent porosity of the nanoparticles. *In vitro* studies have showed the uptake of drug-doped nanoparticles into the cytosol of tumor cells and significant damage to impregnated tumor cells upon irradiation with light of wavelength 650 nm thus silica NTAs can be used for imaging and PDT functions [86]. Mesoporous silica nanostructures consist of hundreds of empty nano/micro channels ormolecular-sieve structures and a large surface area (>900 m2/g), and can encapsulate small molecules via simple physical interaction and control the drug delivery properties. Moreover, the mesopores can be capped or sealed using Au NPs after drug (e.g. PTX) loading to inhibit premature drug release. Au NP capping is designed to be photolabile and can be uncapped to release guest molecules when photoirradiated. Similarly, activatable seals or caps based on QDs, IONPs, courmarin and diethylenetriamine can be also used. Park *et al.* [87] have developed biodegradable luminescent porous silicon nanoparticles (LPSiNPs) and loaded with DOX. An unique feature of these silica NTAs is that they can self-destruct *in vivo* and can be renally cleared in a relatively short period of time which reduce the risk of getting trapped-in healthy organs and causing damages. Furthermore, authors [87] have used these NTAs for tumor imaging and slow release of DOX upon the degradation of nanoparticles.

Liposome NTAs

Liposomes are comprised of closed spherical vesicles which are composed of a lipid bilayer of either synthetic or natural phospholipids with diameters around 100 nm. They have been widely used as drug delivery vehicles as liposomes can encapsulate both therapeutic and diagnostic agents, protect the encapsulated agents from external environments, prolong systemic circulation lifetime of the encapsulated agents, and can be functionalized with various targeting ligands for cell- or tissue-specific delivery [88]. Additionally, liposomes have been used as drug carriers for the cancer therapy [89,90]. Liposomes are reportedly used to encapsule a variety of contrast agents such as super paramagnetic iron oxide NPs (SPIOs) [91,92], gadolinium-based, and manganese-based compounds [93-95] for MRI applications to enhance the contrast in T2-weighted MRI for better *in vivo* visualization. These imaging agents together with the therapeutic agents loaded into liposomes have led to a variety of NTAs. For example, the encapsulation of iron oxide into a cholesterol/DOPE/DSPC liposome to form multifunctional NTAs [96]. Sundararajan *et al.* have developed radiolabelled liposomes by encapsulating ^{186}Re and DOX into liposome interior for the cancer chemoradionuclide therapy [97] and tested in male nude rats bearing xenografts of head and neck squamous cell carcinoma. This liposome formulation showed a prolonged circulation time and decreased liver accumulation. Also the combination of radionuclides with chemotherapeutic drugs has demonstrated real-time imaging and increased efficacy conferred by chemoradionuclide therapy [97]. Additionally, Petersen *et al.* have encapsulated copper-radionuclide (^{64}Cu) in a polyethylene glycol (PEG)-lipid based liposome formulation for PET imaging [98].

Polymeric NTAs

Polymeric NPs have been extensively used for drug delivery applications [99] where the core of polymer nanoparticles can be loaded with a variety of therapeutic or imaging agents. The sustained and controlled release of therapeutic agents or drugs from polymeric NPs have been achieved through different mechanism such as surface or bulk erosion, diffusion through the polymer matrix, swelling followed by diffusion, or stimulation by the local environment [100]. Synthetic polymer with outstanding biocompatibility and biodegradability [101,102] and naturally derived polymers such as chitosan and cyclodextrin [103] have been used to develop biocompatible polymeric NPs based drug delivery systems. Additionally, polymeric NPs have been found to be effective carriers for MRI contrast agents such as SPIOs and Gd-based compounds [104-106]. The mixture of SPIOs and DOX

are directly encapsulated by using amphiphilic block co-polymer, maleimide-PEG-poly(lactic acid) (PLA) and self-assembled to form polymer based NTAs for both the drug delivery and MRI imaging. Surface functional groups such as maleimide groups help the conjugation of cRGD molecules to target αvβ3 integrins [105] and also help to conjugate short peptides containing 10 amino acids to target αvβ6 integrins [106]. The resultant polymeric NTAs are used for the evaluation of pharmacokinetics by real-time MRI in tumor-bearing mice [105-106]. Targeted NTAs are resulted in a higher tumor accumulation and led to the enhanced tumor retardation due to the integrin-mediated endocytosis as compared with non-targeted NPs. Moreover, Pluronic® F-127 micelle are used to form stable formulations of above-mentioned NTAs for simultaneous imaging and therapy. The strategy of encapsulating SPIO-DOX mixture to formulate NTAs is applicable to many existing polymeric NP systems [107].

For radionucleide imaging, radionuclide compounds such as ^{11}C, ^{18}F, ^{64}Cu, ^{76}Br, ^{99m}Tc, ^{111}In and ^{90}Y have been used with a wide range of copolymers such as N-(2-hydroxypropyl)methacrylamide (HPMA) to formulate robust nano-sized delivery systems [108]. Additionally, the fluorescence imaging technique is integrated with polymeric NPs to develop the image-guided drug delivery system to monitor drug pharmacokinetics, intratumoral drug distribution, and drug tumor accumulation in real-time [109]. Peng *et al.* [110] have developed multifunctional polymeric nanotheranostic NPs composed of PEG-polycaprolactone (PCL) di-block co-polymer with a NIR florescent dye (IR-780) for both NIR imaging and PDT. These NTAs are also labelled with ^{188}Re for micro SPECT-guided tumor imaging which heped to observe a preferential tumor accumulation in BALB/c athymic nude mice bearing HCT-116 colorectal carcinoma. The addition of NIR irradiation has enhanced the tumor inhibition as compared with control groups which are without NTAs (which are treated with PBS and NIR irradiation only, or with micelles only). Zhu *et al.* have developed multifunctional BSA-Au nanostars for photoacoustic imaging and X-ray computed tomography which can be used for the therapeutic purposes [111]. Zhang *et al.* [112] have synthesised activatable hyaluronic acid based NTAs for the development of optical/photo acoustic image-guided photothermal therapy for the cancer diagnostics and therapy. These authors [112] have developed a multifunctional activable nanocomposite NTA, Cy5.5–HANP/CuS (HANPC) by loading copper sulfide into Cy5.5-conjugated hyaluronic acid NPs. Cy5.5 fluorescent signal is quenched by CuS inside the particle until the whole nanocomposite is degraded by hyaluronidase (an enzyme) present in tumor, giving strong fluorescence signals delineating the tumor. CuS with strong NIR absorbance is found to be an excellent contrast agent for photo acoustic imaging and an effective photothermal therapy agent. HANPC NTAs are intravenously administered into SCC7 tumor-bearing mice which resulted in high fluorescence and photoacoustic signals in the tumor area over time and maximum signal is seen at the time point of 6 hours[112]. Subsequently the tumors are irradiated with a laserand a good tumor inhibition rate of 89.74% was found on day 5 [112]. Based on the encouraging results, authors [112] have concluded that these HANPC phototherapeutic agents can be effective for the cancer diagnosis and therapy.

Future Challenges and Opportunities

Despite increasing excitement and after a lot of technological development of NTAs and applications, a lot of challenges need to be addressed before realising the full potentials of NTAs. Some of the challenges are as follows:

Clinical Standards: This challenge is one of the most difficult to overcome. To get the regulatory approvals, researchers need to demonstrate the safety and efficacy of NTAs against some gold standards or globally accepted controls. Therefore, there is an urgent need to develop gold standards or acceptable standards to validate the functional integration of imaging and therapeutic efficacyof NTAs.

Toxicity and Safety: The toxicity of QDs and metal particles will be a prime concern from both regulatory point of view as well as from patient's compliance point of view. Despite recent encouraging progresses made in this area, a lot of research work is need to be undertaken to convince regulatory authorities and consumers.

Biodegradability and Biofate: Non-biodegradable nature of CNTs and silica nanoparticles will be a challenge from the consumer's point of view although these nanomaterials can be made safe through various functionalisation methods. Also a lot of studies needed to make sure that these nanomaterials either stay safe in the human body or excreted out quickly without causing any side-effects.

Site Specific Drug Delivery: This area of the research has always inspire researcher to develop site specific drug delivery systems with control over drug release. Particularly, the initial burst release followed by the sustain release of drugs or agents will always be focused in the future development. Moreover, once-a-daily or once-a-weekly dose or implantable drug delivery systems will help reduce the treatment cost and side-effects, and improve the consumer compliance.

Cost of Development and Treatment: The product development cost of nanomaterials particularly that of gold, is generally high. However, economies of scale and new research on the economic production of nanomaterials may help mitigate this issue in future.

Conclusions

Theranostics is a new promising medical paradigm that synergistically utilise therapeutic and diagnostic capabilities of agents for diagnosis, drug delivery or therapy and therapy response recording. This field is further evolving fast into the nanotheranostics which is immensely benefitted by multifunctional nanomaterials that can enhance not only the imaging quality but also the specific targeting of disease sites. Nano-theranostic agents will help reduce toxicity and reduce time of both diagnosis and therapy and help to develop personalised therapies. However there are many technical and non-technical challenges that needed to be overcome to realise full

potentials of this fast emerging field. These challenges will provide many opportunities in future to develop newer, efficacious, cost-effective and safer nano theranostic agents.

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