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REVIEWS

Nanomaterial based Magnetic Resonance Imaging of Cancer

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Abstract | Magnetic Resonance Imaging (MRI) is a widely used non-invasive medical tool for detection and diagnosis of cancer. In recent years, MRI has witnessed significant contributions from nanotechnology to incorporate advanced features such as multimodality of nanoparticles, therapeutic delivery, specific targeting and the optical detectability for molecular imaging. Accurate composition, right scheme of surface chemistry and properly designed structure is essential for achieving desired properties of nanomaterials such as non-fouling surface, high imaging contrast, chemical stability, target specificity and/or multimodality. This review provides an overview of the recent progress in theranostic nanomaterials in imaging and the development of nanomaterial based magnetic resonance imaging of cancer. In particular, targeted theranostics is a promising approach along with its targeting strategy in cancer treatment using MRI and multimodal imaging. We also discuss recent advances in integrin mediated targeted MRI of cancer.

1 Introduction

Among all diseases 'Cancer' is one of the world's leading causes of death today, as is evident from the world cancer statistic as shown in Figure 1.[1] Cancer is defined as a class of diseases characterized by unregulated or uncontrolled cell growth (proliferation). So far, 100 different types of cancers have been classified on the basis of type of cell tissue that is initially involved.[2–4] These overgrown cells harm the body by increasing the interstitial pressure; upon further division in an uncontrolled manner the formation of lumps or masses of tissue called 'tumor' results (except in the case of leukemia where cancer prohibits normal blood function by abnormal cell division in the blood stream). Tumors grow and interfere with the digestive, nervous, and circulatory systems and they can release hormones that alter body function.

Tumor with limited growth that stays in one spot is generally considered to be benign (non cancerous). Tumors which spread through the blood stream and are known to show pathological symptoms are called malignant tumors. The key processes of invasion[5] (destruction of healthy tissue and penetration through lymph) and angiogenesis generate malignancy. The hallmarks of cancer[6] constitute sustained proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis, tumor-promoting inflammation, genome instability and mutation etc. Understanding these became important for developing cancer therapeutics. Fortunately, imaging diagnostics has emerged as a powerful tool for the assessment and detection of tumors. Particularly, integration of molecular biology and imaging techniques is increasingly being used to understand the complexity, diversity and *in vivo* behaviour of cancer.[7] MRI (Magnetic Resonance Imaging), one of the most important techniques, is a noninvasive imaging modality that enables to provide anatomical, physiological and even molecular information of the body. MRI offers high contrast of soft tissues and enough penetration depth to visualize the entire human body. In recent years, significant advances have been made in designing biocompatible, noninvasive and sensitive molecular imaging probes and

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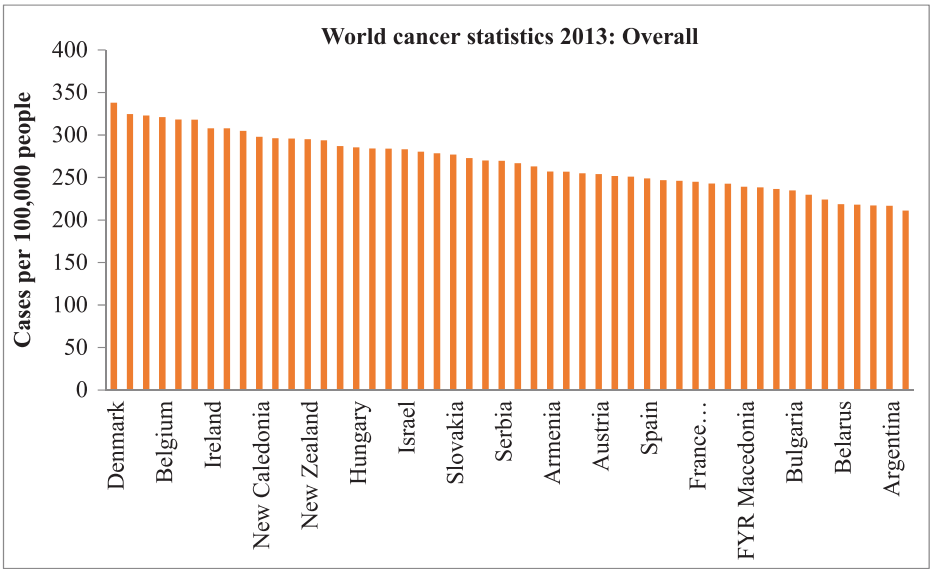

Figure 1: Cancer statistics 2013: Data comparing more and less developed countries.

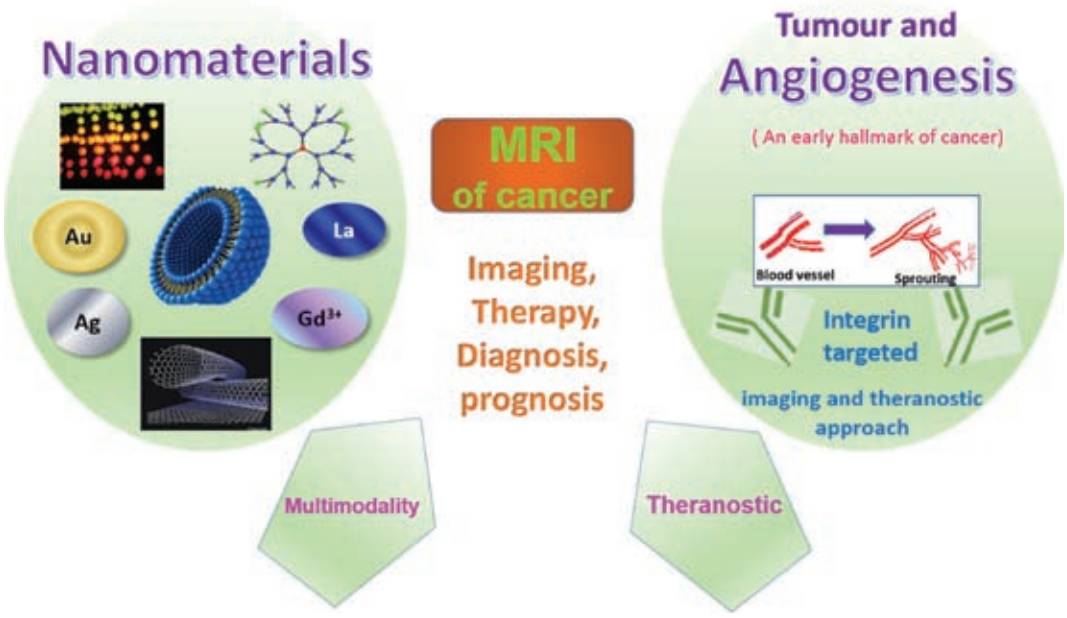

Figure 2: Schematic illustration of nanomaterial based MRI of cancer targeting angiogenesis.

to achieve target specificity various biomarkers are used. Among all the cancer bio-markers that are currently under investigation, integrins are the main focus. Upregulations of these cell adhesion molecules have been found to be tightly associated with a wide range of cancer types, making it a broad-spectrum tumor marker, and they serve as activators for pathological angiogenesis.[8–10] 'Arginine- glycine-aspartic acid' (RGD) is one of the recognition site for few integrins such as $\alpha_v\beta_3$, thus it is used as a targeting agent in many imaging probes to target angiogenesis via integrin.[11] In this review, we discuss various imaging techniques, design of various nanomaterials for MRI imaging, and its application in theranostic and multimodal imaging approach. Towards the end, we will focus on the recent advances in MRI to target angiogenesis in cancer via integrins.[2,12,13] Figure 2 summarizes the same content of the review.

1.1 *Imaging overview*

Biomedical nanotechnology has made a major impact on the development of molecular imaging probes for cancer clinical trials and medical

practices using various nano-platforms.[14,15] In addition to the energy based classification, all current imaging techniques can be grouped on the basis of spatial information that is attained—macroscopic, mesoscopic or microscopic, or the type of information that is obtained—Physiological, cellular, molecular or anatomical. Macroscopic imaging modalities that provide physiological and anatomical information are now in widespread clinical and preclinical use. It includes Single-Photon Emission-Computed Tomography (SPECT), Positron Emission Tomography (PET), fluorescence reflectance imaging, Fluorescence-Mediated Tomography (FMT), Fiber-optic microscopy, Optical frequency-domain imaging, bioluminescence imaging, ultrasound, optical imaging and Magnetic resonance imaging (MRI). All of them provide wealth of information that is highly complementary, and hold much promise as they allow real time visualization of the cellular functions of living organisms and related molecular interaction. Importantly they are noninvasive techniques. PET and SPECT are highly quantitative and sensitive with no penetration limitation, but both make use of ionizing radiation, which is incompatible for frequent imaging, due to potential damage to the living subjects from the cumulative irradiation dose. On the other hand, optical imaging, which is less expensive and user friendly, has the limitation of penetration depth and poor multiplexing, making it less effective for cancer detection and assessment (i.e. it is limited to surface imaging; for example, colon cancer with optical endoscopy, bladder cancer imaging with optical cystoscopy or skin cancer).[16,17] Moreover, PET, SPECT and Optical imaging have low spatial resolution. A completely noninvasive and inexpensive ultrasound anatomical imaging technique is one of the promising tools for the evaluation of tumors. However, the limitations of ultrasound include the dependence on the skill of an operator and limited depth penetration. A problematic issue in clinical settings of ultrasound is to receive an adequate documentation for the comparison of examinations at different time points, and not all regions of the body are accessible to the ultrasound probe,[18] e.g. lung, bone, and brain in adults. Similarly, Computed Tomography[19] has a drawback with respect to its sensitivity. The comparison between different imaging techniques is summarized in Table 1.

An alternative noninvasive and highly sensitive tool is MRI, which is widely used clinically among numerous anatomical techniques to assess tumor growth and for response evolution.[20] MRI offers realistic chances for early detection of neoplastic lesions *in vivo* and it is able to acquire three dimensional topographical information in whole tissue samples, including human soft tissue, and whole animals, at high spatial and temporal resolution.[7,21–27] High spatial resolution of 10–100 μm can be acquired, and it dependent on magnetic field. Currently, US Food and Drug Administration (FDA) has approved 3-Tesla magnetic field strengths, as spatial resolution of human body imaging is restricted to approximately 1 mm. Although tissue MRI is capable of revealing anatomical details in organs, difficulties are seen in differentiation between normal and tumor cells due to small native relaxation differences. To achieve enhanced imaging sensitivity exogenous MRI contrast agents (Gd^{+3}, Iron oxides) have been applied.[25,28]

1.2 *Principles of MRI*

MRI works on the principle of NMR (Nuclear Magnetic Resonance), and it produces contrast in biological systems by measuring the relaxation processes of hydrogen atoms in different microenvironments. Radiofrequency (RF) waves have wavelengths of the order of 1 cm and can penetrate deep in to body, therefore MRI has no limitation for tissue penetration.[29] In the presence

Table 1: Comparison of different imaging techniques.

Modality	Source	Typical probes	Resolution/Sensitivity/Time/Depth
SPECT	γ-ray	^{99}mTc, ^{111}In-labelled compounds	1–2 mm/ 10^{-10}–10^{-11}/minutes to hours/no limit
PET	γ-ray	Radioisotopes (eg. ^{18}F, ^{11}C, ^{13}N, ^{15}O, ^{64}Cu,^{124}I)	1–2 mm/10^{-11}–10^{-12}/minutes to hours/no limit
CT	X-ray	Iodine, Barium sulphate, Gold	50–200 μm/10^{-6}/minutes/no limit
MRI	Radio wave	Paramagnetic (Gd^{3+}), Supramagnetic (Fe_3O_4)	10–100 μm/10^{-9}–10^{-6}/minutes to hours/no limit
Optical Imaging	Light	QDs, NIRF dyes	0.3 μm /10^{-12}/sub seconds to minutes/<10 cm

of an external magnetic field, protons (^{1}H spins) align in one direction (z axis). Upon application of the RF pulse, aligned protons are perturbed and subsequently relax to their equilibrium (original state). There are two independent relaxation processes: longitudinal (T_1) and transverse (T_2) relaxation, which are typically used to generate the MR images.[28] T_1 is the time constant of the exponential recovery process of spin magnetization along the z-axis after an RF pulse. Rapidly relaxing protons (short T_1) recover full magnetization along the z axis and produce high signal intensities. For protons with slow relaxation (long T_1), full magnetization is not recovered before subsequent RF pulses, therefore they produce less signal and result in the so called 'saturation effect'.[30] The time constant of the exponential decay of the transverse magnetization (Mxy) after an RF pulse is known as T_2. It is related to the amount of time for precessing magnetic moments of the protons to become randomly aligned in the xy-plane after an RF pulse, eventually resulting in a net magnetic moment of zero in the xy-plane. A combination of local fluctuations in the magnetic field due to magnetic interactions of neighbouring molecules and macroscopic effects related to inhomogeneity in the external magnetic field causes this dephasing process. When the dephasing time accounts for both intrinsic molecular interactions and extrinsic magnetic field inhomogeneities, it is termed 'T_2^*'.

Signal contrasts can arise in MRI from differences in four basic physical parameters: i) Spin density of the various tissues/fluids being analyzed, ii) T_1 iii) T_2 iv) T_2^*;

Signal intensities of MRI are dependent on T_1 and T_2 relaxation times that are sensitive to the microenvironment. There are few basic MRI pulse sequences as follow:

Spin-Echo: $S = \rho\,(1 - \exp(-TR/T_1))\exp(-TE/T_2)$,
Inversion Recovery: $S = \rho\,(1 - 2\exp(-TI/T_1) + \exp(-TR/T_1))$
Inversion Recovery with SE (180-90-180): $S = \rho\,(1 - 2\exp(-TI/T_1) + \exp(-TR/T_1))\exp(-TE/T_2)$
Gradient Echo: $S = \rho\,(1 - \exp(-TR/T_1))\sin\theta\exp(-TE/T_2^*)/(1 - \cos\theta\exp(-TR/T_1))$,

where ρ = spin density, TR (The Repetition Time), TE (The Echo Time), TI = the Inversion Time, θ = the excitation angle.

The actual contrast $C = |SA - SB|$ achieved between signals SA & SB, will depend on the intrinsic T_1 & T_2 of the two tissues, and on the TR (The Repetition Time) and TE (The Echo Time) parameters used.

Key approaches to achieve the contrast include

- *T_2-weighting*: where a Spin Echo (SE) sequence with 'longish' TE (to magnify T_2 decay differences) and long TR (to minimize T_1 recovery differences) are used.
- *T_2^*-weighting*: where a Gradient Echo (GRE) sequence, with not-so-long TE (to highlight T_2^* effects) and long TR (to minimize T_1 differences) are used.
- *T_1-weighting*: makes use of a GRE or a SE sequence, but with short TE (to make differences in T_2^* irrelevant) and short TR (to make T_1 recovery differences important).
- *ρ-weighting*: using simple density weighting, with no contrast from T_2^*, T_2 or T_1.

Malignant and healthy tissues have minor differences in their overall water content. But they exhibit substantial differences in their T_1's & T_2's, thus MR image contrast is important to differentiate soft tissue from its surroundings. Often exogenous materials, i.e. contrast agents, are employed clinically to enhance the contrast between the tissue of interest and the surrounding tissue. T_1-weighted images illustrate anatomy well and are preferred when a clear image of structure is required[30] and T_2-weighted images produce good pathological information since collections of abnormal fluid appear bright against the normal tissue background.[31] Therefore, there are two classes of MRI contrast agents available, (1) T_1-weighted contrast agents (e.g., gadolinium-(Gd^{3+}) and manganese-(Mn^{2+}) chelates) are paramagnetic in nature, which increase the T_1 relaxation time through coordination with water molecules providing increased contrast, hence also called positive contrast agents, and (2) T_2-weighted contrast agents such as super paramagnetic magnetite (Fe_3O_4) nanoparticles are also called negative contrast agents because they reduce T_2 relaxation times, thus decreasing the MRI signal intensities around them, showing dark images. The efficiency of a contrast agent to reduce the T_1 or T_2 of water protons is referred to as relaxivity. Bae et al. recently investigated dual contrast agent composed of a gadolinium chelate on the polymer coating of SPIONs. This excellent feature allows for the acquisition of both highly detailed T_1 weighted anatomical images and pathologically relevant T_2 weighted images with a single imaging nanoprobes.[32] In addition, multiparametric imaging is possible in MRI wherein Diffusion Weighted Images (DWI) and T_1, T_2 are obtained in one session, each reflecting a different tissue signal.[33] Multimodality imaging takes advantage of

unique strengths of two or more imaging modalities, and compensates the limitations from a single imaging modality. This provides overall structural, functional, and molecular information, offering the prospect of improved diagnostic and therapeutic monitoring abilities.[34]

An excellent combination of nanoscience and nanotechnology has led a step further towards rapid development of novel nanomaterials as MRI contrast agents. Because of their significant role in MRI, excellent reviews on synthesis and applications of nanomaterial based contrast agents have been published.[28,35–38] These nanomaterial based MRI contrast agents have several advantages over conventional MRI contrast agents such as: a) tunable bio distribution and biostability can be achieved by surface modification; b) different degrees of imaging properties and biocompatibility can be adjusted by their designing schemes that include variations in chemical composition, shapes and sizes; c) target specificity can be assigned to them by respective conjugation with biologically interactive molecules like antibodies, peptides, nucleic acids and peptide-mimetics, and these biomarkers help to locate the changes at tumor site over the time; d) most importantly, the ability to design multimodal imaging probes (Figure 3) using different combination of optical and magnetic properties of nanomaterials e.g. MRI/PET, MRI/PET/NIRF, MRI/CT, PET/UCL/MRI etc.[35,39–41]

2 Nanomaterials in Therapeutics

Nanomaterials have been utilized as therapeutic drug delivery vehicles and for treatment of a variety of diseases and disorders.[42] These nanomaterials have emerged as an excellent alternative for drug delivery. Nanostructured materials display three major unique properties not observed in their bulk counterparts. They possess: 1) 'ultrahigh surface effect' allowing for dramatic increase in the number of atoms in the surface,[43] 2) 'ultrahigh volume effect' allowing for light weight of small particles,[44] 3) 'quantum size effect' allowing for nanosize decrease and quasi-discrete energy of electron orbital around the Femi energy level.[45] This increases the band gap between the highest occupied molecular orbital and lowest unoccupied molecular orbital. Therefore, the electromagnetic quantum properties of solids are altered. When the nanometer size range is reached, the quantum size effect becomes pronounced, resulting in unusual optical, acoustic, electronic, magnetic, thermal and dynamic properties. Furthermore the size, surface characteristics and shape of a nanoparticle also plays a key role in its bio distribution *in vivo*.[46]

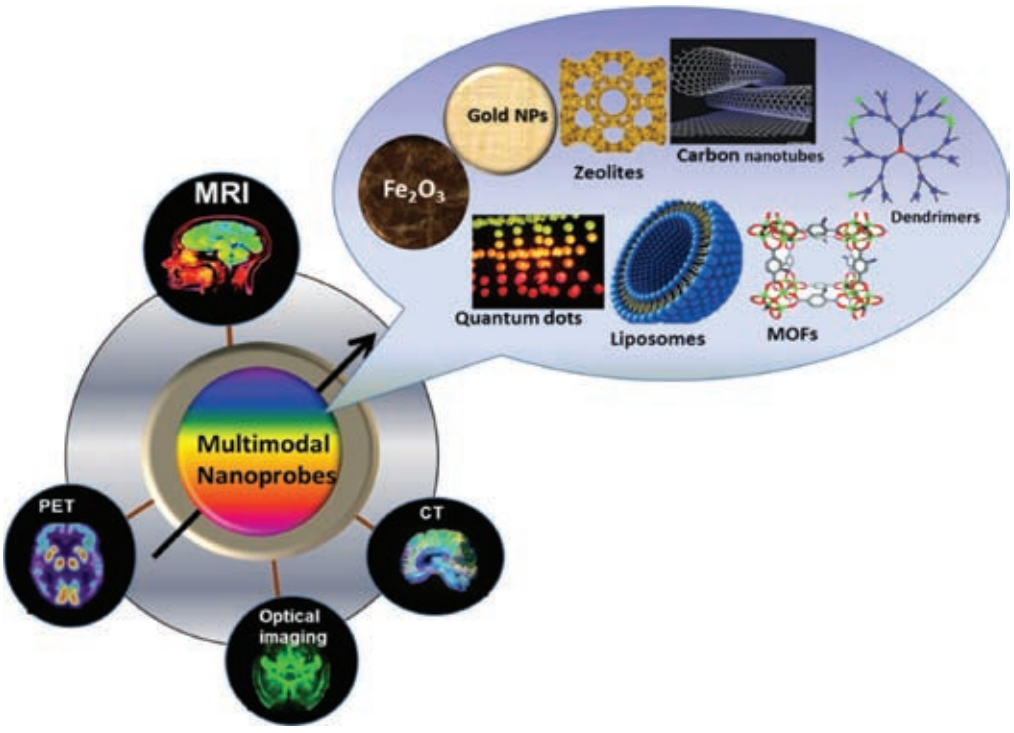

Figure 3: Design of multimodal imaging probes using different combination of optical and magnetic nanomaterials.

There are three main components to an effective drug delivery via nanoparticle:[47] (i) the nanoparticle core material, (ii) the therapeutic agent or drug, and (iii) surface modifiers or functionalization of nanoparticles. Nanomedicine carriers generally have the ability to load either hydrophobic or hydrophilic therapeutics. Thus, suitable carrier materials have to be thoughtfully selected for every therapeutic. However, some carrier materials have both hydrophobic and hydrophilic regions.[48] These materials could be effectively used to design nanocarriers for delivery of multiple drugs. In addition, the nanoparticle core material must be non-toxic and non-immunogenic, and should be easily eliminated from the body to avoid toxic accumulation and side effects.

The most important requirement of an ideal nanomaterials drug carrier is its ability to release the therapeutic drug molecule after the carrier has reached its destination. Surface modification or functionalization of the nanoparticle include both targeting moieties, which assist in accumulation of the carrier in a specific location, and biocompatibility modifiers, which are needed to increase circulation at a specific location.

2.1 *Nanomaterials for cancer imaging*

Many different types of nanomaterials have been developed to provide contrast in medical imaging.[49–51] Some of these materials incorporate an imaging moiety into their design, while others provide contrast as a result of their intrinsic material properties. Multiple imaging modalities can also be implemented into a single nanotheranostic design by incorporating multiple moieties to provide a more complete picture of the disease.

Molecular imaging can identify tumour cell location within the body, and aims to provide information such as metabolism, expression profile, and stage of the disease.[52] Furthermore, molecular imaging can reveal early tumour response to therapy that will aid in improving treatment regimens.[53] A wide range of nanoparticles have been designed to reach tumours. An overview of the different types of nanoparticles[51,54–60] is provided in Figure 4.

Here NPs are broadly classified by the materials they are made of, which include liposomes and micelles, polymers and dendrimers, quantum dots, noble metals, semiconductors, carbon nanotubes and fullerenes, transition metal oxides, metal-organic frameworks, zeolites, mesoporous silica materials and lanthanide series. Although all of these types of nanoparticles have been studied for cancer diagnosis and drug delivery and release, each type of nanoparticle can exhibit different and sometimes unique properties, which make them useful for different applications. Diagnostics may be performed *in vivo* or *ex vivo,* and offer information about a disease's metabolic/biochemical state, genotype, size, location(s), morphology, chemical composition, rate of change, and so forth. A therapeutic improves the outcome of a disease state. Although in the current review, we focus on

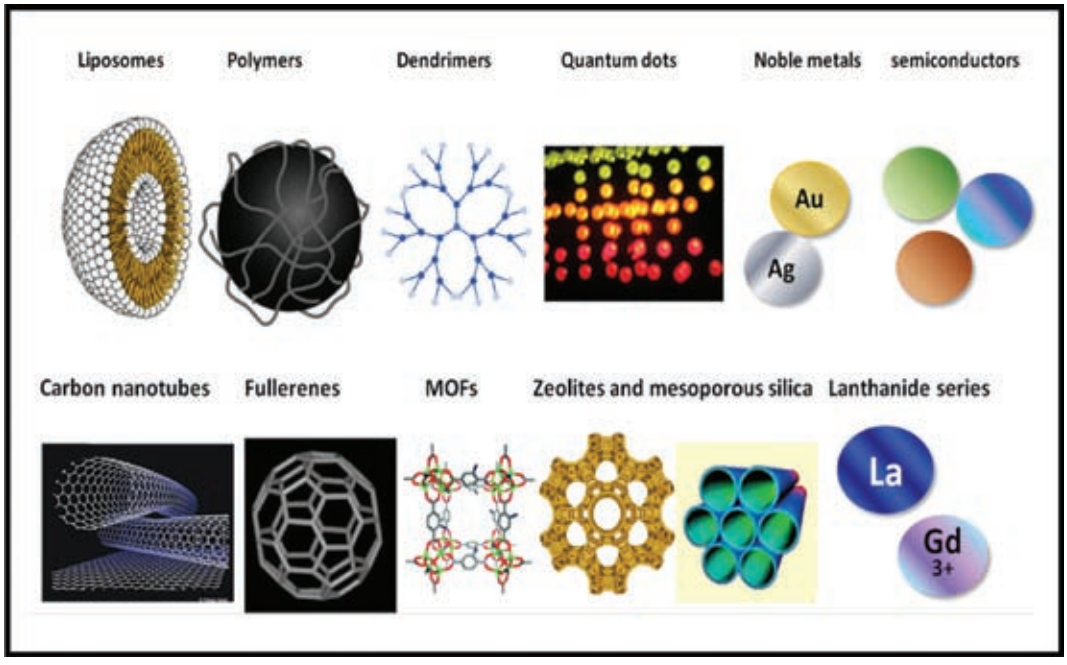

Figure 4: Types of materials used for MRI in cancer diagnosis and treatment.

application of theranostic nanomaterials in cancer biology and *in vivo*, they also have relevance in diabetes and in regenerative medicine.[61]

A brief overview of progress in applications theranostic nanoparticles and nanomedicine in MRI is summarized in Figure 5.

The significant reports are discussed below. Therapeutic nanostructures and imaging nanoparticles have a long history; they have only recently begun to coalesce into the theranostic nanoparticles. In early 1990s, Josephson et al. reported surface functionalized superpara-magnetic iron oxide colloid as MRI contrast agent.[62] They found this colloid as hepatic selective (HS) MR contrast agent. At 20 μmol Fe/kg, the HS MR agent darkened MR images of liver. The HS MR agent exhibited no acute toxicity when injected into rats at 1800 μmol Fe/kg. Originally nanopartciles were used either as therapeutic (delivery) or as diagnostic (imaging) agents. Later nano systems capable of simultaneous therapy and molecular imaging (theranostic) were realized. Subsequently, during the mid 1990s first FDA approved nano-drug came in the market. Doxil,® the first FDA approved nano-drug (1995), was developed in Israel and the USA ~14 years ago when it became evident in a "first in man" (FIM) clinical trial by Gabizon and Barenholz.[63]

There are currently more than 35 FDA-approved nanoparticles (Table 2), with a larger number in preclinical studies for both imaging and therapy.[64–67] Most FDA-approved NPs are used as mechanisms of drug delivery with the exception of MRI contrast agents. The concept of 'theranostic' appeared first time in literature in 2002,[68] although the diagnostic and therapeutic applications of nanomaterials were already studied independently.

Gao et al. in 2004 described development of multifunctional nanoparticle probes based on semiconductor Quantum Dots (QDs) for cancer targeting and imaging *in vivo*.[69] The structural

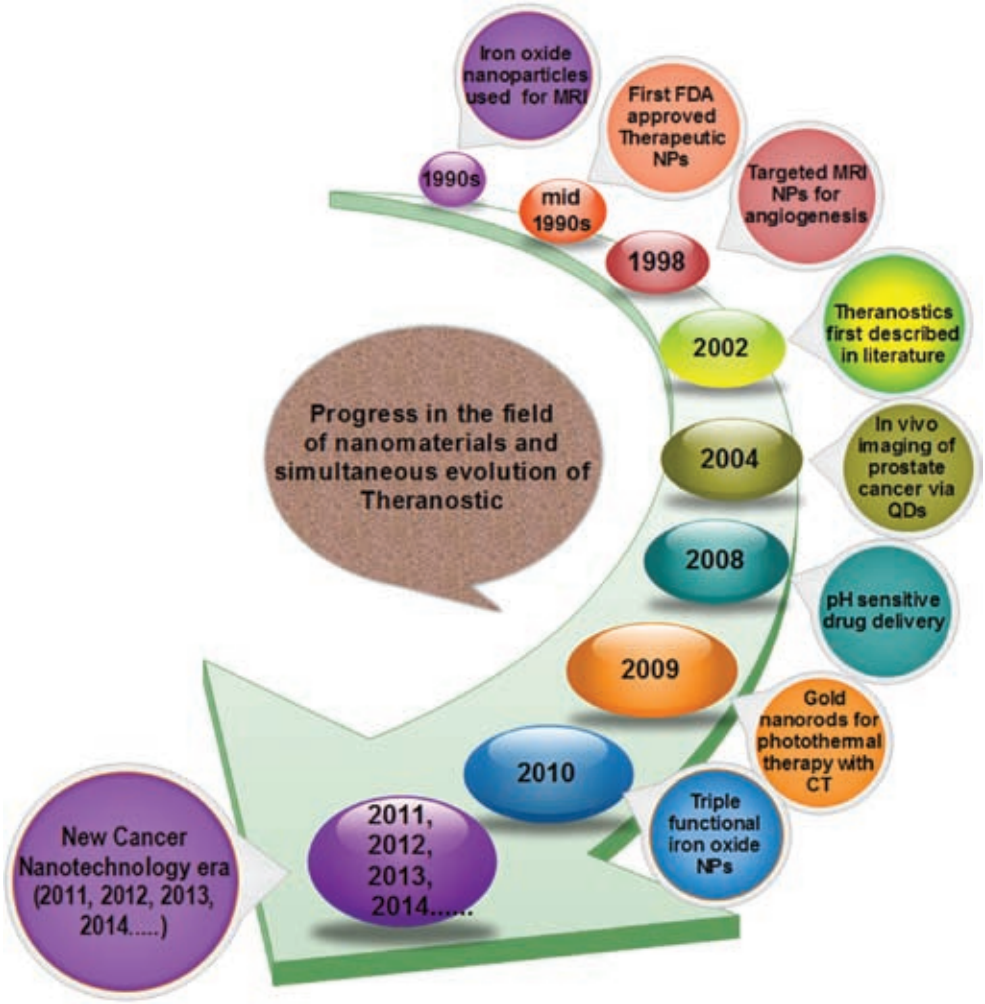

Figure 5: Progress in the field of nanomaterials and simultaneous evolution of theranostics.

Table 2: FDA approved Nanomedicine products.

Product	Type of material	Company	Indication
Doxil[75]	PEGylated liposome	OrthoBiotech	Metastatic ovarian cancer
Abraxane[75]	Albumin-bound paclitaxel particles	Abraxis Oncology	Lung cancer, breast cancer, others
NanoTherm[76]	Iron Oxide NPs	MagForce	Solid Tumor
Feridex IV, GastromarkCombidex (Ferumoxtran-10)[77,78]	Iron Oxide NPs	Advanced Magnetics	Enhanced MRI Contrast
CellSearch® EpithelialCell Kit[78]	Iron Oxide NPs	Veridex, LLC (Johnson & Johnson)	Magnetic Separation

design involves encapsulating luminescent QDs with an ABC triblock copolymer and linking this amphiphilic polymer to tumor-targeting ligands and drug-delivery functionalities. *In vivo* targeting studies of human prostate cancer were carried out in nude mice. They found that the QD probes accumulate at tumors both by the enhanced permeability and retention at tumor sites and by antibody binding to cancer-specific cell surface biomarkers. They achieved sensitive and multicolor fluorescence imaging of cancer cells under *in vivo* conditions using this multifunctional nanoparticle probes. They integrated a whole-body macro-illumination system with wavelength resolved spectral imaging for efficient background removal and precise delineation of weak spectral signatures. Based on their findings they proposed new possibilities for ultrasensitive and multiplexed imaging of molecular targets *in vivo*.

Banerjee et al. in 2008 described targeted anticancer drug delivery over pH-sensitive magnetic nanoparticles for simultaneous imaging and sensing.[70] A pH responsive nanocarrier was synthesized by coupling doxorubicin (DOX) to Adipic Dihydrazide-grafted gum Arabic Modified Magnetic Nanoparticles (ADH-GAMNP) via the hydrolytically degradable pH-sensitive hydrazone bond. The resultant nanocarrier, DOX-ADH-GAMNP, was 13.8 nm in diameter and the amount of DOX coupled was about 6.52 mg g^{-1}. They demonstrated that when excited in the near-infrared region, because of two-photon absorption mechanism, both GAMNP and DOX exhibited fluorescence properties. Their results illustrate coupling of DOX to GAMNP in a reversible self-quenching of fluorescence through the Fluorescence Resonant Energy Transfer (FRET), where in GAMNP acts as donor and DOX as acceptor. They confirmed the pH sensitivity of the nanocarrier by zeta potential and plasmon absorbance in different pH conditions. Their finding of multifunctional nanocarrier was a significant breakthrough in development of a drug delivery vehicle that combines drug targeting as well as sensing and therapy at the same time.

Agasti et al. in 2009 reported photo regulated release of anticancer drugs from gold nanoparticles.[71] They conjugated anticancer drug 5-fluorouracil to the surface of gold nanoparticles through a photocleavable o-nitrobenzyl linkage. The gold nanoparticles serve as both cage and carrier for the therapeutic, providing a nontoxic conjugate that effectively releases the payload upon UV irradiation of long wavelength.

In 2010 Xie et al. reported triple functional iron oxide nanoparticles (IONP) for PET/NIRF/MRI.[41] They used dopamine to modify the surface of IONPs, yielding nanoconjugates that can be easily encapsulated into Human Serum Albumin (HSA) matrices. This novel nanosystem, the HSA coated IONPs (HSA-IONPs) were dually labelled with ^{64}Cu-DOTA and Cy5.5, and tested in a subcutaneous U87MG xenograft mouse model. This nanosystem was investigated for *in vivo* PET/NIRF/MRI tri-modality imaging and ex vivo analyses, and histological to test for *in vivo* behaviour of the nanostructures. They found that the particles have a good retention rate and a high extravasations' rate at the tumor sites from imaging and histological investigations.

Chen et al. (2012) reported pH-responsive MRI, ultrasonography and circumvention of Multidrug Resistance (MDR) in cancer cells using manganese oxide-based multifunctionalized mesoporous silica nanoparticles.[72] They demonstrated a new strategy for highly efficient pH-activatable MRI combined with dual-modality biological imaging and intracellular anticancer drug delivery by constructing PEGylated (the process of covalent attachment of polyethylene glycol (PEG) polymer chains to another molecule) versatile mesoporous nanotheranostics for simultaneous non-invasive cancer diagnosis and efficient chemotherapy. Specifically,

manganese oxide NPs with pH-sensitive dissolving behaviour has been introduced into the mesopores of hollow silica nanocapsules by the in-situ redox reaction. The release of Mn^{II} in acidic condition from MnOx NPs-dispersed mesostructure significantly enhances MRI-T_1 performance of Mn-based contrast agents (almost 11-fold magnitude increase), which is very sensitive to the detection of the acidic tumor microenvironment upon arrival on the tumor sites. The designed multifunctional hybrid NPs have been demonstrated as the efficient contrast agents for ultrasound imaging for the first time. Importantly, the drug released from encapsulated nanocapsules could circumvent the multidrug resistance of cancer cells to restore the anti-proliferative efficacy, and enhance the chemotherapeutic efficiency of anticancer agents due to the unique biological characteristics of NPs such as NPs-mediated endocytosis, intracellular drug release, and P-gp inhibition/ATP depletion in cancer cells. This engineered theranostic agent would greatly contribute to the progress of protocols for efficient cancer diagnosis and therapy.

Gomes et al. reported (2013) an efficient pro-survival/angiogenic miRNA delivery by an MRI-detectable nanomaterial.[73] They used biodegradable nanoparticles (NPs) containing perfluoro-1,5-crown ether (PFCE), a fluorine based compound (NP170-PFCE) with the capacity to track cells *in vivo* by Magnetic Resonance Imaging (MRI) and efficiently release miRNA. NP170-PFCE complexed with miRNAs accumulate within the cell's endolysosomal compartment and interact with higher frequency argonaute2 (Ago2) and GW182 proteins, which are involved in the biological action of miRNAs. The theranostic aspect of their formulation makes it very promising for cardiovascular applications. They demonstrated that the release of miR132 from the NPs increased by 3-fold the survival of endothelial cells (ECs) transplanted *in vivo* and 3.5-fold the blood perfusion in ischemic limbs relative to control (cells transfected with empty NPs). The formulation reported the uses of FDA-approved components, which should facilitate its biomedical translation; the formulation can be prepared with controlled size, can incorporate multiple ligands, and can be monitored by ^{19}F MRI. $^{19}FMRI$ is the ideal tool for non-invasive assessment of cell fate after transplantation, providing positive, quantitative, and background-free contrast.

Tian et al. (2014) reported engineered design of theranostic up-conversion nanoparticles for tri-modal luminescence/magnetic resonance/X-ray computed tomography imaging and targeted delivery of combined anticancer drugs.[74] They developed multifunctional nanoparticles based on NaGdF4:Yb/Er@NaGdF4 core–shell UCNPs, which are not only endowed with up-conversion luminescence (UCL), Magnetic Resonance (MR) and X-ray Computed Tomography (CT) imaging ability, but can also be applied as nanocarriers for targeted drug delivery. They systematically investigated their up-conversion luminescent, magnetic and X-ray attenuation properties. Importantly, for the first time, they reported the controlled loading and delivery of a mixture of chemotherapeutic anticancer drugs, camptothecin (CPT) and doxorubicin (DOX), through UCNPs-based nanocarriers. This is significant given that the combined use of two or more drugs usually exhibits much better therapeutic efficacy than that of a single drug. By conjugating nanoparticles with folic acid (FA) which target folate receptors over expressed on various types of cancer cells, they further demonstrated targeted tri-modal UCL/MR/CT cell imaging and drug delivery with UCNPs. Their results suggest that these nanocomposites are highly versatile and could potentially be used for simultaneous imaging and therapeutic applications.

2.2 *Theranostic nanomaterials*

Theranostics is a newly emerging concept that involves simultaneous implementation of diagnosis as well targeted therapy.[13,79] Theranostic nanoparticles (NPs) contain diagnostic and therapeutic functions in one integrated system, enabling diagnosis, therapy, and monitoring of therapeutic response at the same time. Theranostic nanomedicine is an emerging field that uses nanomaterials to pull together diagnostic insight for well-informed treatment. The fundamental advantage of theranostic nanomedicine is the use of patient-specific test results to tailor a treatment regimen producing improved outcomes, reduced costs, and fewer side effects. A diagnostic improves the knowledge of a disease state.

2.2.1 Liposomes: Liposomes are spherical vesicles that consist of one or more phospholipid bilayers encapsulating water in their interior.[80,81] The phospholipids are arranged so as to form a closed sphere, shielding their hydrophobic tails from the water, thus leaving water in the liposome interior. Drugs can be encapsulated within the liposomes, not only in the aqueous volume but also within the bilayer, which allows drugs of different hydrophilicities to be carried.[82,83]

2.2.1.1 Theranostic applications of liposomes: Several liposomal formulations have met with success over the years in a number of animal tumour

models. The usual lipid prodrug-based liposomes have shown promise in drug and gene delivery. Currently several liposomal formulation are in the clinical practice containing different chemotherapeutics such as doxorubicin (Doxil1/Caelyx1), doxorubicin (Myocet1), daunorubicin (DaunoXome1) and cytarabine (DepoCyte1) for treating ovarian cancer, AIDS-related Kaposi's sarcoma, multiple myeloma, and lymphomas or leukaemia with meningeal spread.[84] Several other liposomal chemotherapeutic drugs containing doxorubicin, annamycin, mitoxantrone, cisplatin, oxaliplatin, camptothecine, 9-nitro-20 (S)-camptothecin, irinotecan, lurtotecan, topotecan, paclitaxel, vincristine, vinorelbine and floxuridine are at the various stages of clinical trials.[84] Moreover, advances with cationic liposomes have led to the successful delivery of small interfering RNA (siRNA).[85] Targeted liposomal delivery has been explored through the use of Low Density Lipoprotein (LDL) particles as well as haloperidol associated 'stealth' liposomes for genetic therapy of breast cancer cells.[3] Drug delivery and imaging has been combined in some studies of murine tumour model. Thermally sensitive liposomes contained doxorubicin and $MnSO_4$, and the paramagnetic properties of manganese, similar to those of gadolinium, were used as a probe for *in-vivo* monitoring of the drug by MRI.[86] The temperature-responsive particles enter the tumour, shatter, and release the $MnSO_4$. This can be observed through the relaxivity of manganese nuclei under the applied magnetic field.

2.2.2 Polymeric nanoparticles: A wide range of natural and synthetic polymers constitute a platform for synthesis of a variety of nanoparticles. Natural polymers such as chitosan, albumin, heparin, dextran, gelatin, alginate, and collagen as well as synthetic polymers, such as PEG, polyglutamic acid (PGA), polylactic acid (PLA), polycarprolactone (PCL), poly-d,l-lactide-co-glycolide (PLGA) and N-(2-hydroxypropyl)-methacrylamide copolymer (HPMA) have been widely used to prepare NPs and encapsulate drugs for cancer therapy.[55,87–89] In many cases the polymeric NPs comprised a hydrophobic core containing the anticancer agent and a hydrophilic surface layer for the stabilization of the NPs in aqueous environment.[88]

2.2.2.1 Polymer based theranostic agents: Polymeric NPs have been loaded with gadolinium complexes or magnetic NPs in order to image cancer by MRI.[90] Traditionally, magnetic NPs have been encapsulated in the core of polymeric micelles. For example, Gao et al. encapsulated magnetic NPs together with doxorubicin in micelles formed from amphiphilic block copolymers of maleimide-terminated poly(ethyleneglycol)-block-poly(D, L-lactide) and methoxyterminated poly-(ethylene glycol)-block-poly(D, L-lactide) copolymers.[91,92] These micelles were functionalized with agents such as cRGD or a lung-cancer targeting peptide (LCP) for active targeting. Sohn et al. designed magneto-fluorescent polymeric NPs based on glycol chitosan conjugated to *N*–acetyl histidine and bombesin, for targeting GRPRs overexpressed in prostate cancer cells.[93] Magnetic NPs coated with oleic acid were incorporated into the polymeric matrix, and the NPs labelled with the near infrared fluorophore, Cy5.5. Gadolinium, a positive MRI agent, was recently incorporated into polymeric NPs as Gd metal organic frameworks (MOFs), constructed from Gd^{3+} ions and organic bridging ligands, such as 1,4-benzenedicarboxylic acid. This offers exceptional MRI capabilities over traditional methods for incorporating gadolinium into NPs using Gd_2O_3, $GdPO_4$, GdF_3, etc. The surface of MOFs is modified by covalent attachment of polymer chains to obtain the polymeric carriers.[94,95]

Liu et al. described multifunctional pH-sensitive polymeric nanoparticles for theranostic in cancer[96] for simultaneous tumor Magnetic Resonance Imaging (MRI) and therapy. The nanoparticles were self-assembled using the multi-block polymer poly (lactic acid)-poly (ethylene glycol)-poly (L-lysine)-diethylene triamine penta acetic acid (PLA-PEG-PLL-DTPA) and the pH-sensitive material poly (Lhistidine)-poly (ethylene glycol)-biotin (PLH-PEG-biotin). The anti-hepatocellular carcinoma (HCC) drug sorafenib was encapsulated inside the nanoparticles. Gd ions were chelated to the DTPA groups, which were distributed on the nanoparticle surface. Biotinylated vascular endothelial growth factor receptor (VEGFR) antibodies were linked to the surface biotin groups of nanoparticles through the avidin linker to form the target pH-sensitive theranostic nanoparticles (TPTN). TPTN exhibited spherical or ellipsoidal shapes, uniform particle size distribution (181.4 ± 3.4 nm), positive zeta potential (14.95 ± 0.60 mV), high encapsulation efficiency ($95.02 \pm 1.47\%$) and drug loading ($2.38 \pm 0.04\%$). The pH-sensitive sorafenib release from TPTN was observed under different pH values (47.81% at pH = 7.4 and 99.32% at pH = 5.0, respectively). In cell cytotoxicity studies, TPTN showed similar antitumor effect against HepG2 cells compared to solubilized sorafenib solution after pre-incubation in acid PBS (pH = 5.0) for 1 h *in vitro* ($P > 0.05$). In *in vivo* anti-tumor studies, TPTN showed significantly higher antitumor effect in H22 tumor (VEGFR overexpressed cell line) bearing mice

compared to the solubilized sorafenib solution (oral or i.v. administration) group ($P < 0.05$). In the MRI test, the T_1 relaxivity value of TPTN was 17.300 $mM^{-1}\ s^{-1}$, which was 3.6 times higher than Magnevist® ($r1 = 4.8\ mM^{-1}\ s^{-1}$). As a positive contrast agent, TPTN exhibited higher resolution and longer imaging time (more than 90 min) in the MRI diagnosis of tumor-bearing mice compared to Magnevist® (more than 60 min). All these results indicated that TPTN was a promising polymer based theranostic carrier, which could be a platform for the development of novel multifunctional theranostic agents.

2.2.3 Dendrimers: Dendrimers are a class of polymeric macromolecules that consist of repeating branching units emanating from a central core.[97,98] The unique properties of these almost monodisperse polymers reflect their compact, treelike molecular structure, providing an arrangement of inner and outer molecular functionalities that is influenced by the solvent environment.[99] Dendrimers can be considered to comprise three structural components: i) the core, which in larger dendrimers is almost completely shielded from the outside by the dendritic branch, ii) the outer shell, which possesses a well-defined microenvironment and is protected by (iii) the multivalent surface, which usually bears a high number of reactive sites. Depending on the generation, the type of branching unit, and the moieties grafted onto their periphery, dendrimers can be prepared with sizes ranging from 1 to 10 nm. Lower generation dendrimers have a flat star fish like shape. As the generation number increases, dendrimers become more spherical in shape. Importantly, in a physiological environment, higher generation dendrimers are stabilized as compact balls. Sixth generation polyamidoamine (PAMAM) dendrimers resemble proteins in size and shape.[100] Due to the many variations possible in the basic framework and the peripheral substituents, dendrimers can be tailor-made for numerous applications, including diagnosis and therapeutic agents.[101–105]

Lim et al. reported MRI contrast agents based on triazine dendrimers with gadolinium.[106] They prepared four gadolinium (Gd)-based macromolecular contrast agents, G3-(Gd-DOTA)(24), G5-(Gd-DOTA)(96), G3-(Gd-DTPA)(24), and G5-(Gd-DTPA)(96), that varied in the size of dendrimer (generation three and five), the type of chelate group (DTPA or DOTA), and the theoretical number of metalated chelates (24 and 96). They also investigated paramagnetic characteristics and *in vivo* pharmacokinetics of all four contrast agents. The DOTA-containing agents, G3-(Gd-DOTA) (24) and G5-(Gd-DOTA) (96), demonstrated exceptionally high r1 relaxivity values at off-peak magnetic fields. Additionally, G5-(Gd-DOTA) (96) showed increased r1 relaxivity in serum compared to that in PBS, which was consistent with *in vivo* images. While G3-(Gd-DOTA) (24) and G3-(Gd-DTPA) (24) were rapidly excreted into the urine, G5-(Gd-DOTA)(96) and G5-(Gd-DTPA)(96) did not clear as quickly through the kidneys. They proposed triazine dendrimer-based MRI contrast agent exhibits high *in vivo* r1 relaxivity, desirable pharmacokinetics, and well-defined structure.

2.2.3.1 Theranostic applications of dendrimers: Dendrimers have also found applications in the diagnostic imaging of cancer cells, such as MRI. Gadolinium ($^{153}Gd^{3+}$) is generally considered as the best magnetic resonance contrast agent, but attempts to conjugate gadolinium to conventional polymers as well as proteins have met with limited success. However, gadolinium-conjugated dendrimers have allowed the selective comprehensive targeting and imaging of tumours.[107] Boron neutron capture therapy (BNCT) has also attracted attention for the effective removal of cancer cells. BNCT employs alpha-particles produced from the stable boron isotope 10B. When activated with low energy (0.025 eV) or thermal neutrons, 10B produces lithium (^{7}Li) nuclei and alpha-particles, leading to impressive degradation of tumour cells within their membranes.[108] Boronated antibody-targeted dendrimers have been designed for the effective BNCT of gliomas in the rat.[108]

Recently Nwe et al. reported panitumumab monoclonal antibody–dendrimer (mAb-Den) conjugates as intravascular contrast agents for site specific imaging, and they chose smaller F(ab0)2 fragment vehicle for targeting of the antigen.[109] They reported that antibody based MRI contrast agents are useful as blood pool agents. They compared their results with macromolecular MRI contrast agents composed of multiple Gd (III) chelates assembled on a dendrimer platform, and their studies revealed that they are much more efficient and effective in modulating and relaxing water protons as compared to a single chelate unit analogue.

2.2.4 Quantum Dots: Quantum Dots (QDs) are light-emitting nanocrystals made from semiconductor materials; QDs are becoming an important class of biomaterials, because they possess unique optical properties that are unavailable in organic dyes or fluorescent proteins, such as being brighter, more photo- and chemical stable and possessing a narrow emission spectrum. QDs are highly fluorescent semiconductor nanocrystals,

which typically range from 1 to 10 nm in size.[110,111] They are usually composed of semiconductor elements from group II–VI, such as CdSe, CdS, and CdTe, group IV–VI, such as PbS, PbSe, PbTe and SnTe, or group III–V, such as InAs and InP.[112] QDs possess broad absorption and narrow emission spectra, and their emission maxima can be tuned between 450 to 850 nm by changing their size. They are extremely bright due to their high extinction coefficient in the visible spectrum (ε up to $10^6 M^{-1}$ cm^{-1}; 10–100 times greater than for organic fluorophore)[113,114] and high quantum yields (typically > 50%).[115] They are resistant to photobleaching and chemical degradation, and more stable than conventional organic fluorophores and fluorescent proteins.

2.2.4.1 Quantum dots as theranostic agents: QDs have been used to develop multimodal imaging probes for detection of tumors via fluorescence-MRI, by conjugating a ligand for Gd^{3+} complexation[116] or directly doping the QD with Mn^{2+} (as $CdSe/Zn_{1-x}$ Mn_x S),[117] or via fluorescence-PET imaging by conjugation of ligands for complexation of ^{64}Cu[118,119] and other radioactive elements. Manganese (Mn)-doped NIRQDs were recently used to image pancreatic tumors in mice by fluorescence imaging and MRI.[120] Mn-doped CdTeSe/CdS nanoparticles with a fluorescence emission around 822 nm were prepared in a one-pot synthesis from manganese acetylacetonate ($Mn(acac)_3$), and were covered with lysine to enhance water solubility. The QDs were further functionalized with the antibodies anti-claudin 4, anti-mesothelin, or anti-PSCA, which are overexpressed in primary and metastatic pancreatic cancers and used for Panc-1 and MiaPaCa pancreatic cancer cell staining and *in vivo* fluorescence imaging. In addition, QDs radiolabeled with 125 m Te (as Cd 125 m Te/ZnS) have been produced and used to assess biodistribution of the QDs with specific targeting agents.[121]

With all the advances already made in Quantum dot nanotechnology with respect to biocompatibility, distribution, metabolism/excretion, safety issues still remain a major concern. New approaches such as the addition of a silica coating or a biocompatible polymer coating have increased the biocompatibility and minimized the toxicity of these ultra-small particles, resulting in more water-soluble and safer formulations.[122] Similarly, several reports have proposed the highly luminescent, cadmium free QD for a better biocompatibility and lesser toxicity in cellular environment.[123] Nevertheless, the safe breakdown and elimination of nanodevices require more comprehensive study.

2.2.5 Superparamagnetic iron oxide nanoparticles: Iron oxide NPs are the most commonly explored members of a broader class of NPs referred to as magnetic NPs, which have attracted great interest because of their potential use in a broad range of applications, including catalysis, data storage, bioseparations, and MRI. Magnetite (Fe_3O_4, ferrimagnetic, superparamagnetic when the size is smaller than 15 nm) and maghemite (γ-Fe_2O_3, ferrimagnetic) proven to be particularly popular for biomedical applications because of their great biocompatibility. Notwithstanding this, there have been recent reports that naked iron oxide NPs could be toxic for neuronal cells, and that they may potentially induce oxidative stress processes in the body.[124,125]

2.2.5.1 Theranostic applications of SPIONS: Iron oxide NPs are popular materials for the preparation of multimodal tumor imaging/therapeutic agents. For diagnosis, radionuclides such as ^{18}F or ^{64}Cu, are loaded on magnetic NPs by complexation to surface-bound organic ligands (DOTA in the case of 64 Cu(II).[126] Iridium complexes are loaded into magnetic NPs for dual-modal luminescent and magnetic resonance imaging, as well as photodynamic therapy. Lai et al. used silane chemistry to coat magnetite NPs with an iridium complex;[127] the complex was reacted with IECTS (silane), mixed with TEOS, and the mixture hydrolyzed over the surface of the NPs. The resulting water-dispersable, multimodal system was used for phosphorescent labelling and to simultaneously induce apoptosis of cancer cells by production of reactive oxygen species, i.e. singlet oxygen ($^1O_2$). MRI is one of the most important applications of SPIONs. The large magnetic moment of SPIONs distorts the local magnetic moment of water molecules in tissues, resulting in enhanced contrast between tumour and normal tissues. SPIONs are readily taken up by the reticulo endothelial system (RES), resulting in the successful detection of smaller tumours.[128] Similarly, the macrophage mediated uptake of SPIONs leads to their accumulation in the lymphatic system and the subsequent detection of lymph node metastasis.[129]

Chen et al. reported multifunctional iron oxide based nanocarriers for drug delivery and dual modal imaging of MRI and Two-Photon Fluorescence (TPF).[130] This nanocarrier consists of silver (Ag) nanoparticles coating onto the surface of Fe_3O_4@C nanospheres in dimethyl formamide (DMF) solution. These nanoparticles were loaded with 997 mg/g of DOX via hydrogen bonding and physical absorption relying on carbon shell through the formation of chemical bonds between carboxyl of Fe_3O_4@C@Ag nanoparticles and hydroxy of

DOX under NIR radiation. Photo-regulated drug, DOX release was realized due to the photothermal effect in localized surface plasmon resonance of Ag nanoparticles to break the chemical bonds. And cells that uptake the DOX loaded nanocarriers are almost normal when incubated in dark, but tend to be apoptotic when cultured under NIR radiation. The Fe_3O_4@C@Ag nanoparticles enable dual-modal imaging of TPF imaging and MRI due to the magnetism of Fe_3O_4 nanoparticles and surface plasmon resonance of Ag nanoparticles, this multifunctional can find applications in magnetic manipulation and thermal therapy. These multifunctional Fe_3O_4@C@Ag nanoparticles exhibit the potential for simultaneous diagnosis and therapy in biomedical-related areas.

2.2.6 Gold nanoparticles: Among the many nanomaterials being developed for applications in medicine, this review will focus on gold nanoparticles (AuNPs) and their potential as tumor sensors, drug delivery agents and enhancers in plasmonic photothermal therapy for the treatment of cancers. The use of AuNPs is gaining popularity in these areas of research for several reasons. Firstly, AuNPs are considered to be relatively biologically non-reactive, and therefore suitable for *in vivo* applications compared to the very toxic cadmium and silver NPs,[129] although various groups are contesting this view. Other advantageous qualities include the strong optical properties of AuNPs due to localized surface plasmon resonance (LSPR),[131] easily controllable surface chemistry that enables versatility in adding surface functional groups,[132] and lastly, the ease in control over particle size and shape during synthesis.[133] AuNPs may be considered to be fully multifunctional, with the possibility of combining different desired functionalities in one molecular-sized package. All these factors contribute to the strong interest and preference for the use of AuNPs over other NPs.[134] Examples of other nanomaterials for biomedical applications that expound on the utilization of quantum dots can be found in literature,[135,136] functionalized fullerene-based nanomaterials[137] and magnetic NPs[138,139] for the diagnosis and treatment of human diseases.

2.2.6.1 Application of gold nanoparticles in imaging and theranostics: While MRI generally makes use of gadolinium complexes as contrast agents, AuNPs have also found applications. These gadolinium chelates, such as gadolinium diethyl triamine penta acetic acid (Gd-DTPA), produce low magnetic fields and have shown to exhibit kidney toxicity.[140] DNA-templated Au-NP chains on the other hand, have shown an increased phagocytosis capability by the 3D cancer cell scaffolds.[141] Although the layer by layer encapsulated AuNP experienced comparatively weaker local magnetic fields, the greater cell uptake of DNA-AuNP was able to produce statistically equivalent image, contrast in T_2-weighted MRI images.[141]

Interestingly, AuNPs have also been reported to possess antiangiogenic property.[142] The exact mechanism of action is not yet clearly understood, but it has been observed that AuNPs bind preferentially to vascular permeability factor/vascular endothelial growth factor (VPF/VEGF)-165, and basic fibroblast growth factor (bFGF) primarily through the heparin-binding domain. This has led researchers to suggest that AuNPs are able to inhibit angiogenesis by preventing the downstream signaling effects of these mitogens on angiogenesis in cancer cells.[143]

Topete et al. reported targeted multimodal chemo- and photothermal therapy combined with optical and magnetic resonance imaging in cancer using polymeric-gold nanohybrids. The multimodal nano probe design involved folic acid (FA)-functionalized, doxorubicin (DOXO)/superparamagnetic iron oxide nanoparticles (SPION)-loaded poly(lactic-*co*glycolic acid) (PLGA)-gold(Au) porous shell nanoparticles (NPs) as potential nanoplatforms.[144] These polymeric-gold nanohybrids were prepared by a seeded-growth method using chitosan as an electrostatic 'glue' to attach gold seeds to DOXO/SPION-PLGA nanoparticles. They studied their physicochemical properties, cellular uptake, and photothermal and chemotherapeutic efficiencies *in vitro* using a human cervical cancer (HeLa) cell line.

2.2.7 Upconverting nanophosphors: Upconverting nanophosphors (UCNPs) are an exciting new class of fluorescent probes for biomedical imaging that are essentially lanthanide (rare earth)-doped ceramic materials. In contrast to organic fluorophores and semiconductor QDs (so called 'down-converters'), UCNPs convert longer wavelength radiation (typically NIR) into shorter wavelength luminescence, i.e., they exhibit anti-Stokes emission.[145] Presently, the two major types of inorganic host matrices used to prepare UCNPs are rare earth fluorides (e.g. $NaYF_4$ and LaF_3) and oxides (e.g. Y_2O_3 and Y_2O_2S).[146] The major appeal of UCNPs for tumor imaging is that these relatively inexpensive low-power NIR diode lasers may be used as the excitation source, which allows for deeper tissue penetration compared to traditional fluorescence imaging as well as higher contrast optical imaging due to an absence of auto

fluorescence and decreased light scattering.[147] Unlike many organic fluorophores, UCNPs are extremely resistant to photo bleaching, and their rare earth components are approximately one-thousand fold less toxic than the heavy metals within QDs.[148] Moreover, the NIR wavelengths the UCNPs are excited at are less cytotoxic than the radiation used for exciting most other fluorophores.[147] UCNPs functionalized with the cyclic RGD peptide have been successfully used for imaging of integrin $\alpha_v\beta_3$-positive tumor cells.[149] Zako et al. prepared silica-coated Er^{3+} doped Y_2O_3 NPs and modified their surface by treatment with a hetero bifunctional PEG derivative, followed by the RGD peptide.[149] Upconversion emission was observed from the NPs for U87MG cancer cells (high integrin $\alpha_v\beta_3$ expression), but not for MCF-7 cancer cells (low integrin $\alpha_v\beta_3$ expression), confirming integrin $\alpha_v\beta_3$-specific binding/uptake.

2.2.7.1 Application of upconverting nanophosphors in theranostics: Xing et al. reported multifunctional nanoprobes (NaY/GdF_4: Yb, Er, Tm @SiO_2eAu), which show narrow size distribution, excellent dispersity in PBS buffer with low cytotoxicity.[150] This multimodal nanoprobe that combines fluorescence, X-ray Computed Tomography (CT) and Magnetic Resonance (MR) imaging, to give three dimensional (3D) details of tissues and cells of high resolution and sensitivity. They report a trimodal imaging probe with uniform size nanoparticles of less than 50 nm of PEGylated NaY/GdF_4:Yb, Er, and Tm @ SiO_2-Au@PEG5000. These nanoparticles demonstrated strong emissions ranging from the visible (Vis) to near infrared (NIR) for fluorescent imaging, T_1-weighted MRI by shorting T_1 relaxation time and enhanced Hounsfield units (HU) value as a CT contrast agent. They optimized the structure based on influence of the distance between the NaY/GdF4:Yb, Er, Tm core and Au nanoparticles (NPs) at the surface. Further, they demonstrated its potential in trimodal imaging for cancerous cells and lesions both *in vitro* and *in vivo*.

2.2.8 Carbon nanotubes and fullerenes: Carbon nanotubes and fullerenes (CNTs) have been extensively investigated for cancer imaging applications.[151–153] Both single-walled and multi-walled CNTs have a high surface area and internal volume for loading of drugs and imaging agents, but alone, CNTs are not soluble in most organic or aqueous solutions. Therefore, surface modification of CNTs is critical for their use in theranostic applications.[154] Polyhydroxy fullerene can be detected using photoacoustic imaging, and used for photothermal ablation therapy after intratumoral injection.[155] Furthermore, multi-walled CNTs can be used for photothermal ablation therapy owing to their release of vibrational energy upon near-infrared light exposure.[156] However, potential toxicity associated with CNTs must be addressed prior to clinical translation.[157–159]

Recently Wu et al. reported preparation of multiwalled carbon nanotube with cobalt ferrite ($CoFe_2O_4$) magnetic hybrids using solvothermal method.[160] The hybrids materials prepared at 180°C were further investigated for biomedical applications based on the superparamagnetic property at room temperature and high hydrophilicity. They observed a high T_2 relaxivity of 152.8 $FemM^{-1}S^{-1}$ in aqueous solutions, a significant negative contrast enhancement effect on cancer cells, and more importantly, low cytotoxicity and negligible hemolytic activity. Doxorubicin, an anticancer drug was loaded onto the hybrids, which was released in a sustained and pH-responsive way. The DOX-loaded hybrids exhibited notable cytotoxicity to HeLa cancer cells due to the intracellular release of DOX. Their results revealed that carbon nanotube and cobalt ferrite hybrids may as effective magnetic resonance imaging contrast agents and anticancer drug delivery systems for simultaneous cancer diagnosis and chemotherapy.

Shi et al. designed fullerenes and iron oxide composites by draping iron oxide nanoparticles (IONPs) onto the surface of fullerene (C60), followed by PEGylation to improve the solubility and biocompatibility of C60-IONP, and obtained a multifunctional C60-IONP-PEG nanocomposite with strong superparamagnetism and powerful photodynamic therapy capacity.[161] They conjugated a new photodynamic anti-cancer drug, Hematoporphyrin monomethyl ether (HMME), to C60-IONP-PEG, forming a C60-IONP-PEG/HMME drug delivery system, which demonstrated an excellent magnetic targeting ability in cancer therapy. They observed a remarkably enhanced photodynamic cancer cell killing effect using C60-IONP-PEG/HMME as compared with free HMME. They demonstrated C60-IONP-PEG as T_2-contrast agent for *in vivo* magnetic resonance imaging. Their work showed C60-IONP-PEG/HMME had a great potential for cancer theranostic applications.

2.2.9 Metal-Organic Frameworks: Metal-Organic Frameworks (MOFs) are nanosized structures comprising metal cations and electron donors such as carboxylates or amines that form coordination bonds and are self-assembled into highly porous materials.[162] They contain organic molecules that impart synthetic flexibility so that the crystalline structure, size, and porosity can be engineered depending on the

combination of organic linker and the metal cation used in synthesis. Furthermore, the metal cation chosen can impart magnetic properties for detection in MRI. For example, gadolinium (Gd) and manganese (Mn) based MOFs have been synthesized for MR and potential multi-modal imaging.[163–165] Iron (Fe) based MOFs are detectible in MRI after intravenous injection into rats, indicating their utility *in vivo*.[166] Their ease in synthesis makes MOFs a promising theranostic agent, but scale-up for mass production and reduction of synthesis times have been difficult.[167] Horcajada et al. reported non-toxic porous iron (III)-based metal-organic frameworks with engineered cores and surfaces, as well as imaging properties, function as superior nanocarriers for efficient controlled delivery of challenging antitumoural and retroviral drugs, that is, busulfan, azidothymidine triphosphate, doxorubicin or cidofovir against cancer and AIDS. In addition to their high loadings, they also potentially associate therapeutics and diagnostics, thus opening the way for theranostic approach.[166]

2.2.10 Zeolites and silica based materials: Nanocontainers based on zeolite L represent a novel class of nanomaterials that could be of potential use in cancer diagnosis and therapy, because they can be heavily loaded with luminescent molecules, photosensitizer and/or radio metals without leakage after locking with stopper moieties.[168] Efficient functionalization of the zeolite surface can be achieved by direct coupling of appropriate molecules via silanol groups or, for example, using click chemistry, allowing for selective targeting of desired systems. The potential use of zeolite L nanomaterials for scintigraphic imaging (loaded with 111 In) and PDT (grafted with phthalocyanines) has been demonstrated.[169,170] Preliminary results suggest the possibility of developing zeolite L nanocontainers suitable for detecting and curing neoplastic tissue. Lo and coworkers very recently described mesoporous silica nanoparticles (MSNPs) for the controlled release of anticancer chemotherapeutics, which feature doxorubicin (Dox) conjugated to the MSNPs channels via acid-labile hydrazone linkages. Upon exposure to the acidic environment of endosomes/lysosomes, Dox is released intracellularly, resulting in inefficient apoptotic cell death.[171] Csajbok et al. reported Gd^{3+} loaded zeolites for potential application as magnetic resonance imaging (MRI) contrast agents.[172] They explained the role of diffusion for the relaxivity by a comparison of the relaxivity of Gd^{3+} loaded zeolite NaY and NaA samples. They suggest that these materials have a potential as T_1 MRI contrast agents at low field, and as T_2 agents at higher fields.

Table 3: Representative theranostic nanoparticles and their biomedical applications.

Nanomaterial	Drug/imaging probe	Madality	Application	References
Liposomes	Doxorubicin/Mn^{2+}	MRI	Murine tumour model	86
Polymeric nanoparticle	Sorafenib/Gd^{3+}	MRI	Antitumor effect against HepG2 cells	96
Dendrimers	Triazine Dendrimers/Gd^{3+}	MRI	MRI Contrast Agents	106
Quantum dots	Anti-claudin 4, anti-mesothelin, or anti-PSCA/Mn^{2+}	Fluorescence imaging and MRI	Pancreatic tumors	120
SPIONS	DOX/Fe_3O_4@C@Ag	MRI, NIRF	Cytotoxicity to HeLa cancer cells	130
Gold nanoparticles	Folic acid (FA)-functionalized, (DOXO)/(SPION)-loaded (PLGA)-Au	Optical and magnetic resonance imaging	Human cervical cancer (HeLa) cell line	144
Upconverting nanophosphors	PEGylated NaY/GdF_4: Yb, Er, Tm @SiO_2-Au@PEG_{5000}	Fluorescenc, CT and MR	Trimodal imaging for cancerous cells	150
Carbon nano tubes	DOX/Carbon nanotube (MWCNT)/cobalt ferrite ($CoFe_2O_4$)	MRI	Cytotoxicity to HeLa cancer cells	160
Fullerenes	HMME/C60-IONP-PEG	MRI/Photo-dynamic therapy	Murine tumor model	161
MOFs	Doxorubicin/Fe(III) based MOFs	MRI	Controlled drug delivery and antitumoural activity	166
Zeolites	Gd^{3+}-doped zeolites	MRI	Contrast agents	172
Silica based nanomaterials	DOX/peptide-modified magnetic graphene-based mesoporous silica	MRI/Confocal microscopy	Glioma therapy	179

Silica NPs (1–10 nm) hold great potential in emerging applications of diagnostic imaging due to their unique properties, such as tunable light emission, high brightness, stability against photobleaching and low toxicity.[173–177] Surface functionalization with amino groups for instance, opens the way for grafting specific biomolecules, and consequently, to achieve active targeting. Initial bioimaging results have shown that these NPs are readily taken up by murine cells, allowing for efficient staining.[178] Wang et al. reported peptide-modified Magnetic Graphene-based Mesoporous Silica (MGMSPI) as a multifunctional theranostic platform.[179] These theranostic agents are advantageous because of excellent biocompatibility, high near-infrared photothermal heating, facile magnetic separation, large T_2 relaxation rates (r2) and a high doxorubicin (DOX) loading capacity. Applications of various theranostic agents in targeted cancer therapy and imaging have been summarized in the Table 3.

3 MRI Based Targeted Theranostic

Targeted theranostic approach offers an excellent efficacy to perform by clustering the nanoparticles at target of cancer imaging. To serve the same purpose many MRI imaging probes are functionalized with required target, and most of the targets are biomarkers like receptors, cell adhesion molecules, nucleotide etc. Patra et al. recently designed the smart cancer theranostic nanomedicine that is highly promising for non-invasive real time diagnosis, targeted therapy and monitoring of the course and response of the action before, during, and after treatment regimen.[180] Potential of these smart biocompatible theranostic micellar nanostructures as a nontoxic, tumour-target specific, tumour-microenvironment sensitive, pH-responsive drug delivery system with provision for early stage tumour sensing, tracking and therapy for cells over-expressed with folate receptors. This development of MRI-visual order–disorder structures for cancer nanomedicine explores a pH-triggered mechanism for theragnosis of tumor hallmark functions (Figure 6). Super Paramagnetic Iron Oxide Nanoparticles (SPIONs) stabilized with amphiphilic poly(styrene)-b-poly(acrylic acid)-doxorubicin with folic acid (FA, a target for folate receptor) surfacing are employed as a targeted theranostic approach to specifically target, diagnose, and deliver drugs via a single nanoscopic platform for cancer therapy. *In vitro* investigation was carried out for the functional aspects of the micellar nanocomposite using human breast SkBr3 and colon cancer HCT116 cell lines for the delivery, release, localization, and

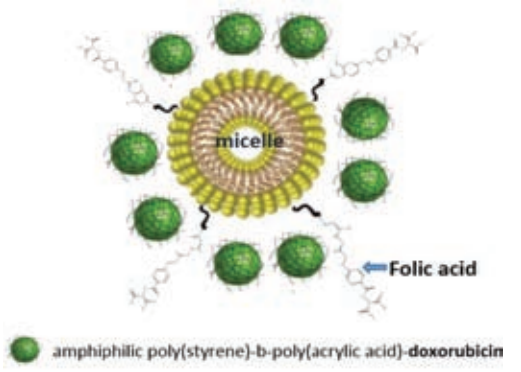

Figure 6: Design of smart cancer theranostic probe.

anticancer activity of the drug. For the first time, pH tunable order-disorder transition of the core-shell structure induced, concentration-dependent T_2-weighted MRI contrast for a monolayer of clustered cancer cells is shown.

3.1 *Angiogenesis: A key process in tumour growth*

The term 'angiogenesis' was first used by the founder of 'scientific surgery' John hunter, (a British surgeon) in 1787.[181] Pathological angiogenesis is an early hallmark of cancer and it is a fundamental requirement for tumour proliferation. Ten decades ago, the observation that angiogenesis occurs around tumours was made.[182–184] The hypothetical concept of production of diffusible 'angiogenic' substances was put forward in 1968.[185,186] In 1971, Judah Folkman the 'father of angiogenesis' proposed that metastasis and growth of tumour are dependent on angiogenesis, and hence blocking angiogenesis could be a strategy to arrest tumour growth.[187] This finding stimulated an intensive search for angiogenic activators and inhibitors. Gullino showed that cells acquire angiogenic capacity on their way to becoming neoplastic from pre-neoplastic.[188] Now there is a wide acceptance for the concept of 'angiogenic switch', it is 'off' when the action of angiogenic activators (pro angiogenic factors) is balanced by that of angiogenic inhibitors (anti angiogenic factors), and is on when net balance is tipped in favor of angiogenesis. Few of them are summarized in Table 4. Regulations of angiogenesis is balanced by secretion of pro- and anti-angiogenic factors from stromal cells, endothelial cells, and cancer cells (Fukumura et al., 1998), the relative contributions of which are likely to change with tumor type and site, as well as with tumor growth, regression and relapse.[189]

'Angiogenesis' is commonly used for the process of vessel growth, but in strictest sense, it denotes

vessel sprouting from pre-existing ones. Physiologically, angiogenesis switch is on in adults during wound healing, corpus leuteum and ovarian follicle development, and in endometrial proliferation.[190] Pathological angiogenesis is a component of many diverse diseases (includes diabetes,[191] atherosclerosis and cancer) that cannot progress without the formation of new blood vessels.[192] Tumour proliferation mainly involves this sprouting of pre-existing blood vessels. Hence 'angiogenesis' is a key stage of cancer and diagnosis of angiogenic events is a potential way to understand and extract strategies for tumour therapy along with the early diagnosis of primary tumour and emerging neoplastic lesions. A brief summary of the role of angiogenesis in cancer and recent advances to develop targeted MRI of tumour angiogenesis (Figure 7) is discussed below. In absence of angiogenesis, solid

Table 4: Angiogenesis activators and inhibitors.

Pro angiogenic factors	Function	Anti angiogenic factors	Function
PDGF-BB[197] and receptor	Recruit smooth muscle cells	Meth-1;[198] Meth-2	Inhibitors containing MMP, TSP and disintegrin
FGF,[199] HGF,[200] MCP-1[201]	Stimulate angio/arteriogenesis	Platelet factor-4[202]	Inhibits binding of bFGF and VEGF
Integrins $\alpha_v\beta_3, \alpha_v\beta_5$ and $\alpha_5\beta_1$[203]	Receptors for matrix macromolecules and proteinases	Angiostatin[204] and related plasminogen kringles[205]	Suppress tumour angiogenesis
Plasminogen activators, MMPs[206]	Remodel matrix, release and activate growth factors	IFN-α-β-γ;[207] IP-10, IL-4, IL-12, IL-1	Inhibit endothelial migration; downregulate bFGF
VE-cadherin;[208] PECAM (CD31)[209]	Endothelial junction molecules	Prothrombin kringle-2;[210] antithrombin III fragment	Suppress endothelial growth
NOS;[211] COX-2[212]	Stimulate angiogenesis and vasodilation	Prolactin[213] (Mr, 16K)	Inhibits bFGF/VEGF
VEGFR,[214] NRP-1[215]	Integrate angiogenic and survival signal	VEGFR-1;[216] soluble VEGFR-1; soluble NRP-1	Sink for VEGF, VEGF-B, PlGF

Abbreviations: VEGFR—Vascular Endothelial Growth Factor (VEGF) Receptors; MMPs—matrix metalloproteinases; NRP-1—neuropilin-1; TIMPs—tissue inhibitors of MMP; IP-10—inducible protein-10; PDGF-BB—Platelet-Derived Growth Factor; NOS—nitric acid synthase; COX—cyclooxygenase-2; FGF—Fibroblast Growth Factors; HGF—Hepatocyte Growth Factor.

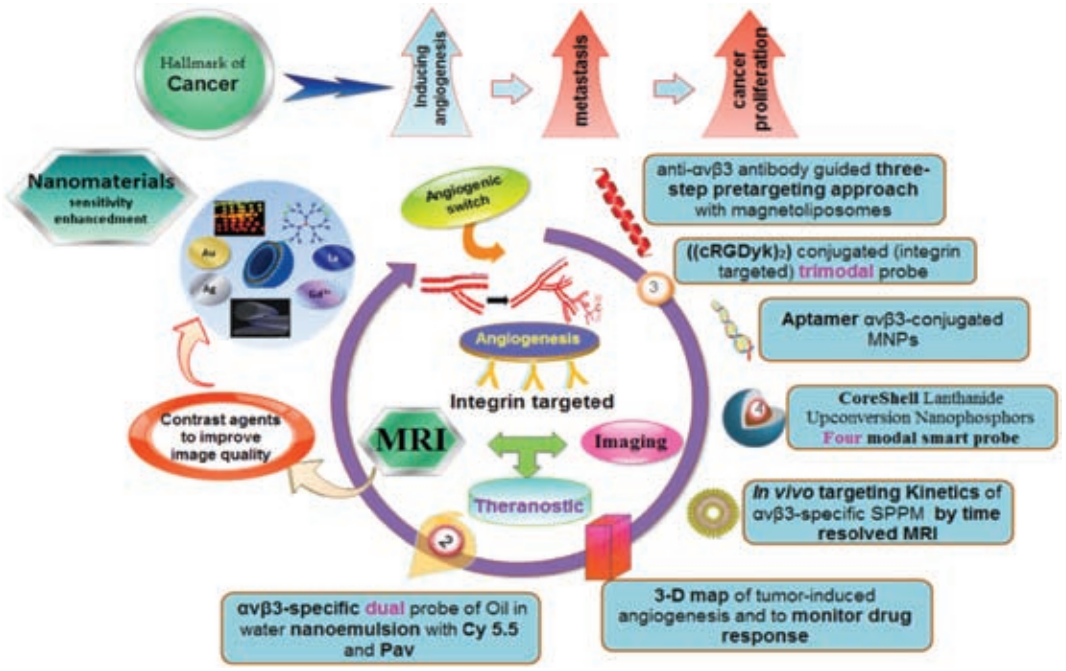

Figure 7: Schematic illustration of recent advances of nanomaterial based integrin targeted MR imaging of angiogenesis.

tumours generally grow larger than 2–3 mm in diameter.[187] Metabolic signals in tumour microenvironment triggers to switch on angiogenic switch, thus tumours start growing in size. It increases the interstitial pressure within tumour, and causes the inhibition of the diffusion of metabolites and nutrients essential for the tumour growth, and a state of hypoxia begins in the cells. Hypoxia increases the transcription of cellular hypoxia inducible factor. Vascular Endothelial Growth Factors (VEGF), a key player of angiogenic process start expressing due to the binding of hypoxia-inducible factors to the hypoxia response elements. Same cellular stimuli trigger expression of a variety of regulating factors (e.g., transforming growth factors-β, acidic fibroblast growth factors, basic fibroblast growth factors and platelet-derived growth factors). These are locally secreted by numerous cells, such as stromal, endothelial and cancer cells.[193,194] Upon endothelial cells activation, proteolytic enzymes such as MMPs (Matrix Metallo Proteinases) and serine proteases are excreted, allowing degradation of basement membrane and extracellular matrix surrounding the vessels.[195] After this degradation, endothelial cells mediated by cell adhesion receptors and they proliferate, migrate until they form unstable micro vessels. To extend, mesenchymal cells release angiopoietin-1, which interact with Tie-2 receptor tyrosine kinase mediating capillary organization and stabilization.[196] The detailed mechanism of angiogenesis is described in many reports.[12,190,192,194]

Thus, the actual proliferation and migration of tumour begin with the expression of various classes of cell adhesion receptors or molecules. Members of the selectine, cadherin, integrin and immunoglobulin families extensively contribute to the tumour vascularisation, not only by participating in those signaling events that regulate the extension and the maturation of neoforming blood vessels, but also by mediating cell-cell and cell-matrix interactions.[217,218]

All these molecules are potential targets for molecular imaging to assessment and diagnosis of various tumours, and have been exploited in different imaging techniques to characterize neoplastic lesions[219–227] Among these, integrin cell receptors are extensively studied. These are heterodimeric transmembrane glycoproteins, which consist of two non-covalently bound transmembrane subunits with large extracellular segments that bind to create heterodimers with distinct adhesive capabilities.[228] As of date, eight beta and 18 alpha, that assemble into 24 different integrins (receptors) such as $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$, $\alpha_9\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$[229] are known. These integrins recognize certain exposed peptide sequences. They act as bidirectional transducer molecules by compromising signals either from the outside to the inside (outside-in) of the cell or vice versa (inside-out), thereby regulating cell adhesion, cell spreading and cell motility.[230] Notably, integrins are known to exist in different conformations:[231]

a. A bent integrin conformation is associated with the low ligand-binding affinity state,
b. An extended conformation is associated with the exposed ligand binding site.

A report from Shattil et al. provides insight into the structure of integrin transmembrane domains, and reveals how the final steps of integrin activation are mediated by integrin binding proteins such as talins and kindlins.[232] They transmit signals through a variety of intracellular protein kinases and adaptor molecules by serving as a site for docking of various kinases and related adaptor proteins; here the β tail serves as a primary site in the formation of focal adhesions. For example, integrins are known to associate physically with

- SFK[233]
- Protein tyrosine phosphatases[234]
- Serine and threonine phosphatases[235]
- C-Src kinase (CSK)
- IRS-1[236] and
- Growth factor receptors,[203] such as FGF receptor (FGFR).[237]

Among 24 integrins, $\alpha_v\beta_3$ is one of the well-studied receptors, which is significantly upregulated on activated angiogenic endothelial cell, but not on quiescent endothelial cells.[8] Many scientific reports showed that integrin expression is correlated with tumour grade,[238,239] and they are a major contributor for the formation of vasculature by supporting migration and survival of endothelial cells.[238] Their activation can be triggered by cytokines of a malignant tumour, and blocking $\alpha_v\beta_3$ integrins inhibits tumour angiogenesis as well as blood vessel formation in *in vivo* models.[240,241] Subsequently, $\alpha_v\beta_3$ might represent a potential target in anti-angiogenic therapy. Hence, $\alpha_v\beta_3$ is counted as a significant biomarker for tumour malignancy. RGD (Arginine- Glycine-Aspartic acid) motif is part of integrin ligands and is recognized by $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins, present in many ECM and some secreted proteins (ligands) such as vibronectin, fibronectin, fibrinogen, laminin, collagen, Von willebrand factor, osteoponin, plasminogen, thrombospondin, prothrombin, MMP-2, laminin, osteopontin, etc.[242] In the early

1970s, E. Ruoslahti discovered RGD as a cell attachment site in fibronectin.[243] The steric conformation of the peptide containing RGD may affect the affinity for their ligands.[244] Besides modulation can be done in the conformational features of the RGD motif and the direct interaction between additional flanking residues of peptide and receptor.[245] RGD recognition is known in bacteria and viruses as well.[246]

By binding to specific integrins and subsequent endocytosis they do enter the host cells.[247] RGD motifs are found to be present in snake venoms, enabling them to affect, for instance, blood coagulation.[248,249] RGD motifs or RGD conjugated many systems have been designed to exploit its affinity as well as the pharmacokinetic properties of the same such as RGD-mediated delivery of small molecule drugs,[250] RGD-targeting of therapeutic peptides and proteins,[251] RGD-peptide mediated delivery of therapeutic nucleic acids,[252] and RGD-equipped imaging agents.[253]

3.2 *Targeting tumour angiogenesis via integrins*

3.2.1 Four-modal imaging probe: Sun et al. developed a lanthanide-based core-shell nanocomposite ($NaLuF_4$:Yb,Tm@$NaGdF_4$(^{153}Sm)) as an optimized four-modal imaging probe with enhanced imaging ability using $NaLuF_4$:Yb, Tm as the core, and 4 nm of $^{153}Sm^{3+}$- doped $NaGdF_4$ (half-life of ^{153}Sm = 46.3 h) as the shell.[254] It was confirmed to be effective and applicable in CT, MRI, SPECT and UCL imaging *in vivo*. Interestingly, the lifetime of upconversion luminescence (UCL) at 800 nm and relaxation rate ($1/T_1$) were at 1044 μs and 18.15 s^{-1} mM^{-1} respectively; however, no significant decrease in the attenuation coefficient was observed, which preserved the excellent X-ray imaging ability. Furthermore, these nanoparticles were applied in tumor angiogenesis analysis by combining multimodality imaging of CT, SPECT, and confocal UCL imaging, which shows its value of multifunctional nanoparticles $NaLuF_4$: Yb, Tm@ $NaGdF_4$ (^{153}Sm) in tumor angiogenesis imaging.

3.2.2 Aptamer for targeted cancer therapy: Lim et al. developed aptamer $\alpha_v\beta_3$-conjugated magnetic nanoparticles ($Apt_{\alpha v\beta 3}$-MNPs) to enable the assessment of physicochemical properties and cyto compatibility nanoparticles and utilizing them for precise detection of integrin expressing cancer cells using magnetic resonance imaging.[255] The term aptamer is derived from Latin root '*aptus*', means 'to fit'. The single stranded DNA or RNA oligo nucleotides (typically 20–100 in length) that fold into specific 3D structures bind to target molecules with high affinity. These highly specific, stable with nuclease resistant serve as an attractive tool for use in a wide array of applications for targeted cancer therapy and diagnosis. For this report, magnetic nanoparticles ($Apt_{\alpha v\beta 3}$-MNPs) were prepared by modifying integrin $\alpha_v\beta_3$-targeting aptamer ($Apt_{\alpha v\beta 3}$) to produce a particle with high ability to detect integrin $\alpha_v\beta_3$ expression in cancer cells during angiogenesis using T_2-weighted MR imaging. $Apt_{\alpha v\beta 3}$-MNPs exhibited an efficient targeting ability with high magnetic sensitivity with no cytotoxicity *in vitro*/*in vivo* studies, thus demonstrating the superb performance of $Apt_{\alpha v\beta 3}$ as a targeting vector having potential for accurate tumour diagnosis and therapy.

3.2.3 Pre-targeting approach: Yan et al. evaluated the tumor angiogenesis targeting efficacy of the anti-$\alpha_v\beta_3$ antibody guided three-step pretargeting approach with magnetoliposomes.[90] The pretargeting approach is based on the avidin-biotin system, and the authors have exploited the high specificity and strong affinity ($Ka = 10^{-15}$ mol/L) of avidin (or streptavidin [SA]) for biotin to improve the sensitivity and specificity of MR imaging. 'Three-step pre-targeting' approached is approved by Paganelli G, Magnani P, Zito F et al. (1991 and 1994), and in Yan et al. report (2013) MR imaging was performed on MDA-MB-435S breast cancer-bearing mice by intravenous administration of biotinylated anti-$\alpha_v\beta_3$ monoclonal antibodies (first step), followed by avidin and streptavidin (second step), and by biotinylated magnetoliposomes or magnetoliposomes in the targeted or nontargeted group, respectively (third step). The modification of polyethylene glycol and liposomal bilayer protected Fe_3O_4 cores from uptake by macrophage cells, hence achieved a greater signal enhancement along the tumor periphery, occupying 7.0% of the tumor area, compared with 2.0% enhancement of the nontargeted group ($P < 0.05$). The specificity of $\alpha_v\beta_3$ targeting was assessed by histologic examinations, showed that the targeted magnetoliposomes colocalized with neovasculature. Thus, the strategy of an anti $\alpha_v\beta_3$ antibody-guided three-step pretargeting approach using superparamagnetic, less cytotoxic and biocompatibility SPION-based biotinylated magnetoliposome is an effective means for sensitive detection of tumor angiogenesis, and may provide a targetable nanodelivery system for anticancer drug.

3.3 *RGD based advances*

In RGD based targeted imaging and delivery of therapy, nanocarriers like liposomes, nanoparticles, micelles, etc. can be grafted at their

surface with a targeting ligand such as an RGD-based sequence to provide the following advantages: i) *Passive targeting*—nanocarriers of size 20–400 nm perform passive targeting of tumours via the so-called enhanced permeability and retention (EPR) effect;[256] ii) *Longer accessibility of the ligand to target*—renal filtration is avoided due to size of these nanocarriers, leading to prolonged blood circulation times and longer accessibility of the ligand to target receptors within the tissue;[257] iii) *Active targeting*—RGD-based nanocarriers may specifically guide drug payloads to tumour angiogenic endothelial cells by the binding of the RGD peptide to $\alpha_v\beta_3$ overexpressed by these cells, allowing 'active targeting' of the tumors;[11] and iv) *Internalization*—These can be internalized via receptor-mediated endocytosis, which is not possible with single peptide constructs or with non-targeted nanocarriers, helping in intracellular delivery of drugs to cancer cells.[244] There are two types of RGD peptides i.e. Linear RGD and Cyclic RGD that are in practice to study the conjugation with nanostructures and its application in cancer treatment. Among them, linear RGD peptide is known to be highly susceptible to chemical degradation, whereas cyclic peptides are more stable, more potent, and more specific, because of rigidity that is conferred by cyclization that improves the binding properties of RGD peptides.[245] Receptor selectivity, receptor affinity and other biological properties could be influenced by the nature of residues flanking the RGD sequence, and the binding specificity is altered by fourth amino acid in the linear RGD peptides. In case of cyclic peptides, few more flanky amino acids are added to RGD the sequence to build a ring system, thus it offers the possibility to present the RGD sequence in a specific conformation for a selected integrin.[245] In particular, since past couple of decades, there is much of advancement by many research group in exploiting the peculiar features of nanomaterials. However, interesting advances like 3D map constructions give the real picture of development of neoplastic cells,[258] and an excellent report of time resolved MRI gives quantification of targeting kinetics and effect of drug payload at tumour site.[259] A lot of attention has been paid to RGD based integrin targeted imaging approach due to its potential therapeutic ability. Thus, nanosystems with RGD motif are of great interest, and by further conjugation with required functionality can gain an excellent design of targeted multimodal probe.

In the same context, our group has reported reversible, spontaneously self-assembled nanotubular structure of fragment of IGFBP-$2_{(249–289)}$ (Figure 8), which contains RGD motif in the sequence.[260] Three Cysteine residues are mainly responsible for this reversible self-assembly and the same cysteines can also be used to ligate the gold Nps in different fashion, further conjugation with MRI contrast agent will provide a nice platform for dual modality probe for integrin targeted imaging of cancer angiogenesis.

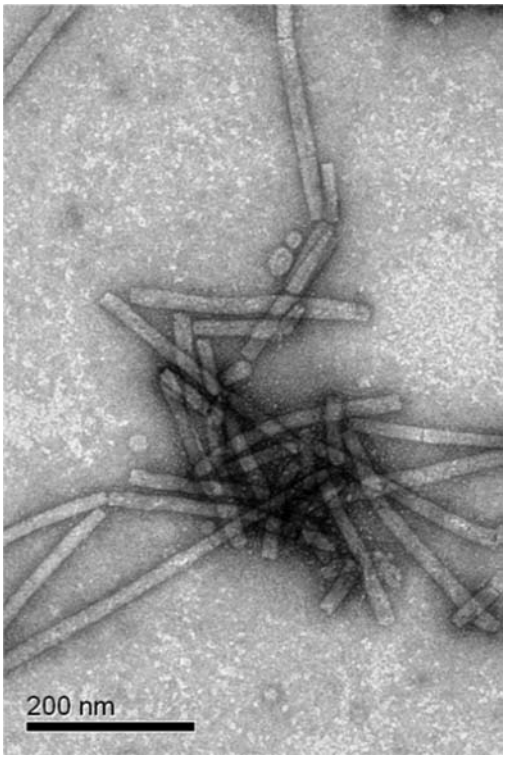

Figure 8: TEM of IGFBP-$2_{(249–289)}$ nanotubes.

Consideration of IGFBP-$2_{(249–289)}$ nanotubes as a targeting agent gains support from many reports on concept of multimeric RGD.[245,261,262] These multimeric RGD nanosystems provide locally enriched concentration of RGD, thus showing higher binding affinity towards integrins and more cellular uptake of respective RGD conjugated nanosystem. Though the binding between RGD peptides to its receptor is also gets affected by the spatial alignments of the peptides.[263]

3.3.1 Quantitative measurements of integrin targeting kinetics: Kessinger et al. demonstrated the feasibility to quantitatively measure the targeting kinetics of cancer-specific superparamagnetic nanoprobes to their biological targets *in vivo*.[259] In particular, kinetics of cRGD-SPPM targeting to $\alpha_v\beta_3$ integrins on the tumor endothelium was evaluated in tumor-bearing mice using T_2^*-weighted time resolved (TR-MRI) sequence. $\alpha_v\beta_3$-specific accumulation of cRGD-SPPM nanoprobes in tumor vasculature was observed

at early times with similar kinetic constants in three distinctive tumor xenograft models (A549, MDA-MB-231, and U87). This data indicates that broadened tumor specificity can be achieved in targeting angiogenic vasculature of tumors where vascular-targeted nanoprobes are less dependent on the specific tumor types, and for the first time that specific vascular targeting can be observed in as early as the first 10 minutes post-injection of nanoprobes (although not surprisingly due to the direct blood-endothelium contact). These results provide useful mechanistic insights for the development of future vascular-targeted nanoprobes to capture the early events of receptor targeting kinetics (e.g. activatable nanoprobes)

3.3.2 Construction of 3D map for assessment of tumour angiogenesis: Schmieder et al. developed an approach to construct a three-dimensional (3-D) map of tumor-induced angiogenesis to quantify and characterize spacial distribution of angiogenesis, and to monitor the effects of drug therapies on angiogenesis in the MDA-MB-435 xenograft mouse model.[258] They designed a perfluorinated nanoparticle loaded with gadolinium ions, which boost magnetic resonance imaging (MRI) signals, and then coating this nanoparticle with a peptide that targets angiogenesis, i.e. RGD containing peptide. For the sake of comparison, the authors also prepared an identical nanoparticle but coated it with a related peptide RGS that does not bind to $\alpha_5\beta_1$ integrin. They also prepared a third nanoparticle coated with a small organic molecule that binds to both $\alpha_5\beta_1$ integrin and $\alpha_v\beta_3$ integrin $\alpha_5\beta_1$ ($\alpha_v\beta_3$)-targeted fumagillin to monitor the antiangiogenic response. Tumor-bearing mice were imaged with MR before and after administration of either $\alpha_5\beta_1$ (RGD) or irrelevant RGS-paramagnetic nanoparticles and 3D reconstructions of $\alpha_5\beta_1$ (RGD)-signal enhancement revealed a sparse, asymmetrical pattern of angiogenesis along the tumor periphery, which occupied <2.0% tumor surface area. $\alpha_5\beta_1$ ($\alpha_v\beta_3$)-targeted fumagillin nanoparticles were less effective ($P > 0.05$). 3D reconstructed map of angiogenesis is useful for characterizing tumors with sparse neo-vasculature that are unlikely to have a reduced growth response to targeted antiangiogenic therapy.

3.3.3 Integrin targeted multimodal MRI of tumour: Chen et al. reported a novel RGD-IONP conjugate with excellent tumor integrin targeting efficiency and specificity as well as limited RES (reticuloendothelial system) uptake for molecular MRI.[264] Coating of ion oxide nanoparticles (IONPs) with a PEGylated amphiphilic triblock copolymer, makes them water soluble and function-extendable. It was further conjugated with integrin $\alpha_v\beta_3$ targeting cyclic RGD peptide, c(RGDyK), along with a NIRF dye IRDye800, were covalently coupled onto the triblock copolymer coated IONPs (TPIONPs) to enhance the tumor-targeting capability. The pharmacokinetics and targeting specificity of this nano conjugate were evaluated both *in vitro* and *in vivo*. Gianella et al. (2012) discovered a flexible and unique multimodal theranostic nanoparticle platform of 50 nm, based on oil-in-water nanoemulsions and carrying iron oxide nanocrystals for MRI, the fluorescent dye Cy5.5 and Cy7 for NIRF imaging and the hydrophobic glucocorticoid Prednisolone Acetate Valerate (PAV) for therapeutic purposes.[265] Target specificity was obtained by functionalizing these nanoemulsions with $\alpha_v\beta_3$-specific RGD peptides, revealing the elevated uptake of by endothelial cells. Simultaneous detection using MRI and NIRF imaging showed significant nanoparticle accumulation in the tumors, while tumour growth profiles revealed a potent inhibitory effect in all of the PAV nanoemulsion-treated animals as compared to the ones treated with control nanoemulsions, the free drug, or saline. Lee et al. synthesized evaluated highly qualified $NaGdF_4:Yb^{3+}/Er^{3+}$ UCNPs as a trimodal upconversion probe PET/MR/luminescence with specific tumor angiogenesis-targeting properties.[11] A dimeric cyclic RGDyk ($(cRGDyk)_2$) peptide was conjugated to $NaGdF4:Yb^{3+}/Er^{3+}$ UCNP along with optimized polyethylene glycol (PEG) molecules, and was consecutively radiolabeled with ^{124}I using a tyrosine residue of ($(cRGDyk)_2$) peptide. Evaluation of this multimodal UCNPs was done in cell cultures and in living mice bearing U87MG human glioblastoma tumors to investigate the feasibility of multimodal imaging using cancer targeting to $\alpha_v\beta_3$ integrin.

4 Future Perceptions

The review explains distinguished sites of nanomaterial designs and excellent features of MRI, which provides for integrating MRI with other imaging techniques to generate efficient multimodal probes, hence acquire maximum data for study, diagnose and treat one of the life threatening disease, i.e. cancer. Development in the field of nanomaterials contributes to a great degree. Targeted theranostic nanomaterials prepend this tremendously with the provision of an effective assessment of tumour and its response to the therapy. Researchers across the globe have been successfully exploiting the peculiar features of nanomaterials adding up to the advancements,

particularly in the last couple of decades. RGD based targeting theranostics are very promising in cancer diagnosis and treatment. Researchers should exploit the multimodal approach, and incorporation of recognition sites like RGD, to develop many more reliable targeted theranostics.

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