

Imaging applications of nanotechnology in cancer

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Abstract Consider a single agent capable of diagnosing cancer, treating it simultaneously and monitoring response to treatment. Particles of this agent would seek cancer cells accurately and destroy them without harming normal surrounding cells. Science fiction or reality? Nanotechnology and nanomedicine are rapidly growing fields that encompass the creation of materials and devices at atomic, molecular and supramolecular level, for potential clinical use. Advances in nanotechnology are bringing us closer to the development of dual and multi-functional nanoparticles that are challenging the traditional distinction between diagnostic and treatment agents. Examples include contrast agents capable of delivering targeted drugs to specific epithelial receptors. This opens the way for targeted chemotherapy which could minimise systemic side-effects, avoid damage to benign tissues and also reduce the therapeutic treatment dose of a drug required. Most of the current research is still at the pre-clinical stage, with very few instances of bench to bedside research. In order to encourage more translational research, a fundamental change is required to consider the current clinical chal-

lenges and then look at ways in which nanotechnology can address these.

Keywords Nanotechnology · Nanoparticles · Imaging · Cancer

Introduction

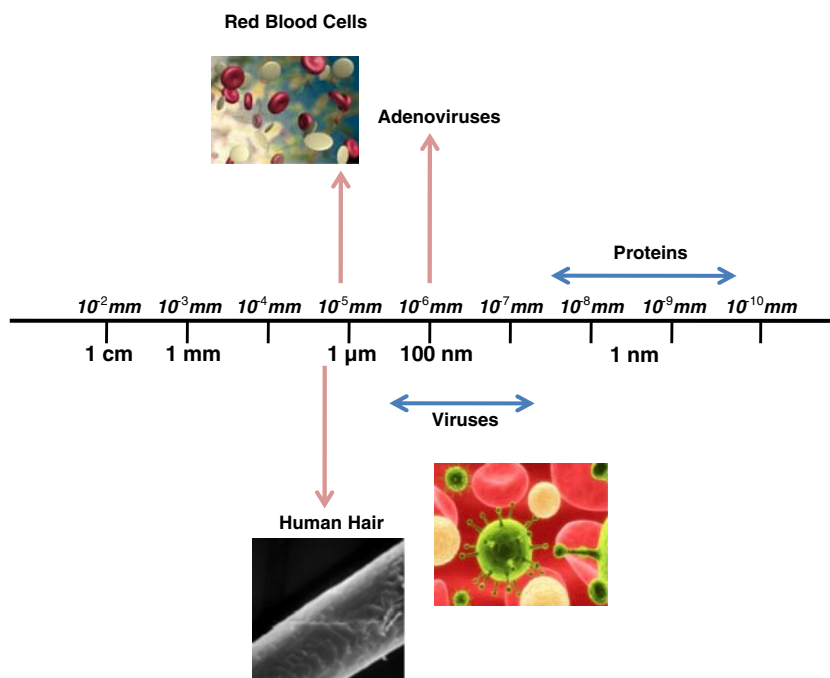
The modern treatment of solid cancers has now become more tailored to the individual patient and to specific tumor types. Tumor markers are now routinely used to decide about suitability for specific medical therapies. Surgery has also become more tailored and in general, more conservative. Advances in imaging techniques allow accurate mapping of lesions improving preoperative planning and patient selection. However, there are significant limitations since existing imaging modalities utilize non-specific contrast agents. For example, iodine-based contrast agents used for computed tomography (CT), can cause significant toxicity. The use of radioisotopes for nuclear imaging exposes patients and healthcare workers to radiation, is heavily controlled by legislation, and provides poor spatial resolution. Nanotechnology may be the key to overcoming some of these limitations.

Nanotechnology was first proposed by the Nobel Prize winner Richard Feynman in 1959 [1]. It is a rapidly growing field that encompasses the creation of materials and devices at atomic, molecular and supramolecular levels. On the metric scale, a nanometer is one-billionth of a meter. Nanoparticles are structures ranging in size from 1 to 100 nm (Fig. 1). Nanoparticles show unique size-dependant physical and chemical properties, which can be optical, magnetic, catalytic, thermodynamic, and electrochemical [2]. These particles have great potential for clinical use, and

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Fig. 1 Particle size comparison

the National Institute of Health (Bethesda, MD, USA) has referred to this area as nanomedicine. The National Institute of Health raised the profile of nanomedicine by allocating funding of \$1.5 billion to nanotechnology in 2009. It is estimated that the global market for nanotechnology could be \$1 trillion within the next decade.

Nanoparticles with a hydrodynamic diameter of less than 5 nm quickly extravasate across the endothelium and have short blood circulation times. Nanoparticles smaller than 6 nm in size, undergo glomerular filtration and renal clearance. Nanoparticles over 8 nm, and those with specific surface properties such as charge and hydrophobicity, are phagocytosed by liver Kupffer cells and undergo clearance via the biliary system [3]. Some small nanoparticles escape opsonisation by the reticulo-endothelial system (RES) and can be large enough to be retained within the systemic circulation. These characteristics, along with the enhanced permeability and retention effect (EPR) demonstrated by Maeda in 2001, leads to longer circulation times of these particles in the body [4]. EPR arises as a result of the production of vascular endothelial growth factor (VEGF) by the tumor. VEGF promotes disorganized angiogenesis leading to the production of “leaky” blood vessels with permeable walls [5]. Many tumors lack effective lymphatic drainage. Both these factors lead to the retention and accumulation of nanoparticles, 10–100 nm in size, at the tumor site.

The large surface area of nanoparticles enables them to accommodate several different functional groups on this surface. By conjugating different functional groups such as

diagnostic (magnetic) and therapeutic (chemo) agents on to the same particle it is possible to create dual or multifunctional nanoparticles, capable of diagnosis and treatment simultaneously [6].

There have been previous attempts at combining imaging with therapy. Radio-immunoconjugates were considered, but they were found to have low tumor uptake, dose-limiting toxicity and exposed patients and medical personnel to ionizing radiation [7]. Cancer nanotechnology is now emerging as a new field of interdisciplinary research, cutting across the science disciplines of biology, chemistry, engineering and medicine. It is likely to evolve into a novel clinical diagnostic and therapeutic field, of clinical nanotechnology.

Any new intervention or technology needs to be ultimately evaluated in the clinical setting to determine its ability to improve both length and quality of life. Nanotechnology is no exception and potential new applications will need to be evaluated within rigorous clinical trials. To identify possible clinical applications, it is important to focus on the areas where clinical or radiological challenges in management exist and then look at ways in which nanotechnology can help address these.

Potential clinical application

Nanotechnology could potentially have several clinically useful applications. Most studies focus on in vitro and in vivo animal experiments, but there are a number of

preclinical and clinical applications that are currently being identified. Targeted drug delivery systems using nanoparticles provide several advantages over conventional antibody guided therapy. Firstly, the drug load delivered to the target can be increased as several targeting ligands can be attached to a single particle [8] and many drug molecules can be contained within the microstructure, known as nanovectors. Nanovectors are able to carry multiple types of drugs, which would result in targeted combination therapy. Their ability to integrate allows access through biological barriers, leading to greater drug loads reaching the target cells. Liposomes are a good example of a nanovector, as they use the leaky nature of tumor vasculature to concentrate at these sites. Liposome-encapsulated doxorubicin has been used for the treatment of Kaposi's sarcoma, breast and ovarian cancers [9].

There are several other examples in the literature of emerging nanosystems for drug and gene delivery. Yang et al. in 2007 [10] demonstrated a multifunctional nanosystem, consisting of magnetic nanoparticles for magnetic resonance imaging (MRI), could be used *in vivo* and *in vitro* to inhibit tumor growth using doxorubicin linked to specific antibodies. The progress of these multifunctional particles could also be followed to their target sites using MRI. The antibody moieties ensure specific targeting of cancer cells limiting any damage to the surrounding normal cells. Nanosystems can also be designed to introduce genes into cells as demonstrated by Medarova et al. [11]. They developed a dual-purpose probe for the *in vivo* transfer of siRNA and simultaneous imaging of its accumulation in cells using MRI and near-infrared optical imaging. Their success in establishing proof of principle has far-reaching consequences as it is applicable to any disease amenable to manipulation at the gene expression level [11].

The heating component of nanotechnology consists of thermal ablation therapies. The goal of thermal ablation is to provide a lethal dose of heat to a prescribed tissue volume with little damage to the surrounding tissue [12]. Thermal ablation may prove to become an alternative to surgery in cancers that are inaccessible or in patients who are unsuitable for surgery. *In vitro* studies carried out on breast cancer cells have shown that nanoshells can be used to destroy tumor on exposure to near infrared light [13]. Nanoshells fall within the range applicable for the EPR effect, and thus should accumulate in most tumor types following intravenous injection. The efficacy of photothermal ablation following systemic delivery of nanoshells has been evaluated in a mouse model. Tissues heated above the thermal damage threshold display coagulation, cell shrinkage, and loss of nuclear staining, all indicators of irreversible thermal damage [13]. However, nanoshells are non-biodegradable and concerns about potential long-term side effects may hold back clinical application.

Application of nanotechnology to cancer imaging

The application of nanotechnology to cancer imaging is subdivided into two main areas: (1) nanodetection for sensing protein and cancer cells and (2) nanoparticle or nanovector formulation for high-contrast imaging [8, 14].

Nanoparticles have been successfully used to selectively tag a wide range of medically important targets, including bacteria, biomarkers and individual molecules such as proteins and DNA [2]. Nanoparticle devices are currently being developed for the early detection of cancer cells in body fluids such as blood and serum. Capturing circulating tumor cells is of great interest and is currently included in the design of large adjuvant and neo-adjuvant clinical trials [15]. Current systems are limited in their ability to accurately select and collect sufficient numbers of these cancer cells for analysis. On average there are only 1–2 cancer cells per milliliter of blood. The nanoparticle devices being evaluated are conjugated with cancer-specific antibodies or ligands that may improve the yield of cancer cells captured [16].

The field of imaging nanoparticles also offers fluorescent nanoplatforms on which targeting agents such as antibodies or ligands can be conjugated. It is possible to image a single cell or an entire organism *in vivo* [17]. Dual-mode nanoparticles can be imaged with MR and optical imaging. As the same particle is evaluated with different imaging modalities, accuracy is increased due to cross evaluation.

More than 60% of patients with breast, lung, colon, prostate, and ovarian cancer have hidden or overt metastatic colonies at the time of presentation [18]. Currently MRI, positron emission tomography (PET), single photon emission tomography, and computed tomography (CT) are non-invasive imaging modalities used for cancer detection in humans. Advances in nanotechnology may lead to a nanoparticle-based tumor-targeting contrast agent that will provide enhanced sensitivity and specificity for tumor imaging [19], enabling earlier detection of metastases. However, this could lead to over diagnosis and overtreatment in some instances, and this potential harmful effect should therefore be considered from the outset. This point is exemplified by work done by Harsinghani et al. [20, 21], which showed that nanoparticle-enhanced MRI improved identification of lymph node metastasis in solid tumors. It was able to identify histologically positive nodes outside the usual field of resection, effectively “upstaging” a proportion of cancers that would have otherwise been identified as node negative. If used at face value without recognizing that this is currently a research-tool, this may lead to an increase in the amount of tissue removed by the surgeon potentially increasing patient morbidity, without a clear benefit.

There is a lot of experimental work being carried out on possible cancer imaging techniques using nanotechnology.

Most of this work is at the preclinical stage with only a couple of studies reaching clinical trials. Most of the existing nanoparticles are not unifunctional and current studies focus on multifunctional capabilities of nanoparticles. The most commonly used nanoparticles in biomedical research are reviewed below and summarized in Table 1.

Preclinical studies

Quantum dots

Quantum dots (QDs) are nearly spherical crystalline semiconductor particles, typically less than 10 nm in diameter, containing roughly 200–1,000 atoms [22]. These semiconductors are characterized by composition-dependent band gap energy. The band gap energy is the minimal energy required to excite an electron from its ground state to a higher level. As the electron relaxes and returns to the ground state, a photon is emitted, leading to a visible fluorescence. The band gap energy is dependent on the size of the semiconductor particle. The optical characteristics of QDs can thus be tuned by adjusting their size [23]. Increasing size also improves penetration depth and reduces background fluorescence at near infrared (NIR) wavelengths.

QDs demonstrate improved signal brightness, up to 10–100 times brighter, when compared with other fluorescent proteins and organic dyes [22]. They also display greater resistance against photobleaching [24] (providing long term stability to the probes), enabling investigations to be carried out over time. In addition, simultaneous excitation of multiple fluorescence colors via a single light source is possible [25].

In order to prolong circulation time QDs can be coated with polyethylene glycol (PEG). PEG-coated QDs are large enough not to be filtered out by renal filtration and small

enough to slow down opsonization and RES uptake [26]. This prolongs their time within the systemic circulation to approximately 3 h.

Optical imaging using QDs is highly specific but is limited by the general drawbacks of optical imaging, including limited depth of tissue penetration and the lack of anatomic spatial information [22]. In order to improve spatial resolution and penetration, QDs have been coupled with paramagnetic gadolinium [27], to enable magnetic resonance (MR) imaging. Significant T_1 contrast enhancement was seen on MR *in vitro* with these combined particles. The *in vivo* imaging potential of these dual-function particles is uncertain due to the instability of the lipid coating used to incorporate the gadolinium on to the QD [27]. However, a new class of QDs have been synthesized using manganese. These particles are intrinsically both fluorescent and magnetic [28] therefore allowing dual imaging. Studies on biocompatibility and potential uses are currently being carried out on this new class of nanoparticles.

It is also possible to form multifunctional nanoparticles by attaching the relevant moieties on to QDs. For example a nanoparticle composed of a QD, a cell targeting ligand and a therapeutic molecule. This combination would form a multifunctional particle able to carry out targeted imaging and therapy. QDs have also been shown to provide a robust scaffold for the delivery of siRNA and imaging [29]. *In vivo* studies have shown that QDs could be utilized to follow the migration of metastatic tumor cells [30]. Multiphoton excitation studies by Voura et al. demonstrated that metastatic tumor cell extravasation could be tracked by using QDs and fluorescence emission-scanning microscopy after tissue resection [30]. The ability to detect several signals from different markers on the same tissue sample simultaneously, is another potential advantage of QDs. Streptavidin conjugated QDs were successfully used to detect immunohistochemical targets in formalin fixed, paraffin embedded tonsil tissue sections [25]. Up to 5

Table 1 A summary of current nanoparticles

Name	Size	Composition details
Quantum dots	2–10 nm	Colloidal fluorescent semiconductor nanocrystals. Central core consists of elements from groups II–VI of the periodic table
Dendrimers	<15 nm	Highly branched synthetic polymers with a layered architecture, consisting of a central core, an internal region and several terminal groups
Magnetic nanoparticles	10–20 nm	Spherical nanocrystals with Fe^{2+} and Fe^{3+} oxide core surrounded by dextran or PEG (polyethelene glycol) molecules, with agglomerates typically 30–150 nm in diameter
Gold nanoparticles	<50 nm	Can be prepared into different geometries: nanospheres, nanoshells, nanorods or nanocages
Carbon nanotubes	<100 nm	Coaxial graphite sheets
Liposomes	50–100 nm	Phospholipid vesicles. Classified by size and the number of layers: multi-, oligo- or uni-lamellar

signals were detected simultaneously on the same tissue specimen.

QDs have limited tissue penetration and lack anatomic spatial resolution, when used for imaging [22], but their main drawback is toxicity. QDs contain toxic heavy metal atoms (e.g., cadmium, mercury, lead), the commonest being cadmium. Oxidative degradation of cadmium-containing QDs leads to the release of cadmium ions, which then bind to sulfhydryl groups on intracellular proteins, disrupting the function of many subcellular organelles [23, 31]. In 2007, Yang et al. studied the pharmacokinetics of QDs in mice. The concentration of cadmium in the kidneys and liver reached 10% and 40% of the injected dose, respectively. There was no fecal or urinary excretion noted within the first 28 days [32], suggesting permanent tissue binding. This highlights the potentially serious toxicity of QDs and at present, they are not ready for clinical evaluation. Future studies will also evaluate the feasibility of protecting the surface of QDs with an outer shell to reduce toxicity.

Core-shell silica particles—C-dots

C-dots are primarily composed of silica, which is non-biodegradable [33]. They are believed to be a safe alternative to QDs. C-dots combine organic fluorophores with a silica core-shell. Limited brightness, environmental-dependant quenching and photobleaching are a few limitations of organic fluorophores, but these are overcome by covalent binding to the silica found in their cores [33]. Choi et al. demonstrated that 30-nm C-dots (injected IV) were excellent imaging agents (multiphoton microscopy) for sentinel lymph node detection due to their bright and stable fluorescence [33].

Gold

Gold is resistant to corrosion, is non-biodegradable, and demonstrates low toxicity and conformational flexibility. These properties make it a very desirable metal for use in medical applications. Gold is also able to accommodate conjugation of proteins on to its surface readily. Gold nanoparticles, less than 50 nm in size are very versatile and can be prepared with different geometries, such as nanospheres, nanoshells, nanorods or nanocages [2]. Kim et al. proposed a biocompatible polymer-coated gold nanoparticle as a potential CT contrast agent [34]. The gold nanoparticle was modified with PEG to prevent uptake by the RES. Animal *in vivo* studies demonstrated that the gold nanoparticles remained in the blood stream for at least 4 h with no appreciable loss of contrast during this time. They were able to achieve good discrimination between normal and hepatoma cells in rats [34]. No apparent toxicity was observed, even at levels of 1 mg/ml, a much higher

concentration than is predicted to be encountered in clinical use.

Carbon nanotubes

Formed of coaxial graphite sheets rolled up into cylinders, carbon nanotubes (CNTs) are less than 100 nm in size. Carbon nanotubes are strong and have good heat and electrical conduction properties. They can be single- or multi-walled [2]. CNTs are generally used for the detection of biomarkers. Recent work done by Liu et al. demonstrates that radiolabeled single-wall carbon nanotubes (SWNTs) can be detected by PET scanning [35]. When conjugated with PEG, the SWNTs are able to circulate in the blood stream for longer and avoid uptake by the reticuloendothelial system, showing similar behavior to other PEGylated nanoparticles [35]. The same study also demonstrated that SWNTs conjugated with arginine-glycine-aspartic acid (RGD) peptide were able to target integrin-expressing tumor cells in mice.

Liposomes

Liposomes are self assembling, spherical, closed colloidal structures composed of lipid bilayers that surround a central aqueous space [19, 36]. Measuring 50–100 nm, they can also be described as phospholipid vesicles. Liposomes are classified by size and the number of layers they contain, i.e., multi-, oligo- or uni-lamellar [2]. Liposomes have already been used for drug delivery in oncology. Due to their amphiphilic nature they are able to transport both hydrophilic and hydrophobic drugs such as doxorubicin, cisplatin, daunorubicin, and taxol. Liposomes have excellent circulation, penetration and diffusion properties, owing mainly to their biochemical composition [2].

Low-density lipoproteins (LDLs) are naturally occurring nanostructures that provide a mode of transportation for cholesterol in mammals [37]. Being about 22 nm in size these particles transport cholesterol to cells that express the LDL receptor (LDLR). They consist of a hydrophobic core surrounded by a phospholipid monolayer. Once LDL binds with LDLR, it gets internalized by endocytosis. Once internalized, LDLR dissociates from LDL and returns to the cell surface to bind further LDL, forming an efficient system for the delivery of cholesterol. Several tumors including colon, prostate, adrenal, breast, and lung tumors are known to overexpress LDLR [38]. In a study done by Corbin et al. [39], an amphiphilic gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA) chelate was intercalated into LDL phospholipid monolayer, in order to deliver Gd(III) to LDLR-expressing tissues. Subsequent *in vivo* studies done in nude mice with human hepatoblastoma G₂ xenografts showed signifi-

cant contrast enhancement on MRI 24 h after administration. This opens up the possibility of using Gd-labeled LDL as a MRI contrast agent for *in vivo* tumor detection. Integrating imaging and therapeutic agents into liposomes means these particles could also be used to create multifunctional nanoparticles.

Dendrimers

These nanoparticles are highly branched synthetic polymers that are usually less than 15 nm in size [2]. Their architecture consists of a central core, an internal region and numerous terminal groups that determine the characteristics of the dendrimer. Dendrimers have a unique feature, an interior void space, which is capable of hosting guest molecules, such as drugs [40]. Dendrimers lack immunogenicity which limits detection by the immune system allowing longer circulation times [40]. As chemical modifications of their multiple terminal groups can easily be achieved, these particles have the potential to become excellent therapeutic and imaging agents.

In 2004, Kobayashi et al. designed a generation 6 polyamidoamine (PAMAM-G6) dendrimer and used it as a contrast agent for micro-MR lymphangiography. Clear visualization of the lymphatic system was seen in normal mice as well as on the xenografted breast tumor models [41]. A gold dendrimer entrapped with gold nanoparticles (Au DENP) was demonstrated by Shi et al. in 2007 [42]. Au DENPs are easy to visualize due to high electron density. The authors demonstrated that Au DENPs can be covalently linked with targeting ligands (folic acid) and imaging molecules (fluorescein isothiocyanate) for cancer cell (KB cells—a human epithelial carcinoma cell line) targeting and imaging. Functionalized Au DENPs were found to be able to differentiate cancer cells from surrounding cells or tissues [42]. It is thought that drug molecules could also be conjugated onto Au DENPs allowing the dual function of imaging and treatment.

Metal nanoshells

Nanoshells consist of a dielectric spherical core nanoparticle such as silica, surrounded by an ultra thin, conductive metal shell, typically gold [12]. By varying the composition and the dimensions of these two layers, nanoshells can be designed and fabricated with plasmon resonances from the visible to the infrared regions of the spectrum [12]. This allows the design of particles that are able to match the wavelength required for a specific application. The plasmon resonance of a particle determines its optical absorption and scattering; this can be tuned by changing the size of the core and the thickness of the metallic shell. It is possible to design nanoshells to absorb

as well as scatter light from the near infrared (NIR) wavelength region. The NIR wavelength provides the maximum penetration of light through tissues. The ability to control their wavelength provides the opportunity for these nanoparticles to be utilized as both diagnostic and therapeutic agents. It is also possible to easily attach antibodies and other targeting ligands onto the surface of nanoshells to enable molecular specific imaging [12]. As is the case with other nanoparticles, polymers such as PEG can also be attached onto nanoshell surfaces to improve circulation times and to enhance biocompatibility.

Nanoshells can be designed to absorb NIR light and generate localized heat. This feature can potentially be harnessed for thermal ablation therapy in cancer treatment. Thermal ablation will offer a minimally invasive alternative to surgery and will be most useful in situations where surgical excision is not a possibility, either due to the location of the tumor or patient factors. Thermal ablation would involve injecting nanoshell suspensions into tumor sites followed by exposing the area to near infrared wavelengths. This process would be monitored by MRI thermal imaging. The tissues containing the nanoshells become heated rapidly once exposed to near infrared light [13].

Nanoshells have also been used as a contrast agent for photoacoustic tomography—a hybrid imaging modality that uses light to heat elements within tissue. The thermoelastic expansion that results from the rapid heating of the tissue generates photoacoustic waves that can be detected with an ultrasonic transducer. *In vivo* studies have been done on cerebral vascular imaging. NIR absorbing, PEG coated gold nanoshells were used. The results showed a greater than 60% increase in optical absorption of the brain tissue leading to a greater contrast in the images generated [43].

Magnetic nanoparticles

Superparamagnetic iron oxide nanoparticles (SPIONs) consist of an iron oxide core and hydrophilic coating. The core can consist of Fe_3O_4 (magnetite) or $\gamma\text{-Fe}_2\text{O}_3$ (maghemite) [44]. The main advantage of magnetic nanoparticles is their ability to be visualized by MR imaging, SPIONs are able to establish magnetic field gradients that alter proton relaxation properties, thus providing a source of contrast for magnetic resonance imaging (MRI). Other advantages include their ability to be guided to target sites by means of an external magnetic field, and their ability to be heated in order to provide hyperthermia for cancer therapy [45]. Their ability to magnetize essentially vanishes in the absence of an applied magnetic field [46, 47]. SPIONs are biocompatible as they are degraded into nontoxic iron ions *in vivo* and disperse well in physiolog-

ical mediums. As a contrast agent SPIONs' efficacy is not influenced by its surroundings [46] unlike some other contrast agents.

In 2007, Lee et al. [48] reported anti-biofouling polymer coated, thermally cross-linked superparamagnetic iron oxide nanoparticles (TCL-SPIONs) for use in cancer imaging via dual imaging MR and optical techniques. Cy5.5 (optical dye) was conjugated on to the TCL-SPION to enable optical imaging. Even though the particles did not contain any targeting ligands, high doses were seen at tumor sites on T₂ weighted MR imaging and on ex vivo fluorescence imaging. This study proved that TCL-SPIO nanoparticles were effective at detecting tumors in vivo by dual imaging, even though it did not have any targeting ligands attached. This preferential concentration at tumor sites was explained by the EPR effect. Further studies were carried out on animal models by Yu et al. [45] using TCL-SPION as a drug delivery system for doxorubicin. Tumor-bearing mice (Lewis lung carcinoma cells were injected subcutaneously into the mice) were injected with doxorubicin or TCL-SPIO nanoparticles conjugated with doxorubicin (Dox@TCL-SPION). The mice injected with Dox@TCL-SPION showed a 63% inhibition of tumor growth compared to 38% in the free doxorubicin group. Apparent toxicity was found to be significantly higher in the latter group. Toxicology studies were carried out on liver and spleen samples from the mice. White cell counts were also looked at to prove that Dox@TCL-SPION was non-toxic and did not lead to myelosuppression. Dox@TCL-SPION proved to be a model drug delivery system that targeted the tumor and carried out exceptional antitumor effects without any systemic side effects.

Integrins are membrane proteins that are responsible for binding cells to the extra cellular matrix. Integrins are located on the cell membrane and are involved in signal transduction between the extra cellular matrix and the cell [49]. In tumors, integrins, such as $\alpha_v\beta_3$, are over expressed. RGD, arginine-glycine-aspartic acid, is a tripeptide sequence found to readily bind to integrins by Pierschbacher and Rouslahti in 1984 [50]. By combining the RGD peptide moiety to a fluorescently labeled cross linked iron oxide (CLIO) nanoparticle, Montet were able to track the binding of the nanoparticle to $\alpha_v\beta_3$ -expressing BT 20 human breast cancer cells and 9 L rat gliosarcoma cells [51].

Magnetic nanoparticles have also been used in the development of dual purpose probes for in vivo transfer of siRNA [11]. Since RNA interference (RNAi) has emerged as a therapeutic option for cancer, it has become important to find a method to detect siRNA delivery into cells and monitor the effects of silencing. The particles demonstrated by Medarova et al. have been used to deliver siRNA to the cells and simultaneously image its accumulation within the tumor cells [11]. They used dextran-coated supra-

paramagnetic nanoparticles conjugated with Cy5.5 dye (for NIRF optical imaging) and a synthetic siRNA duplex targeting gene of interest. The delivery of the probe into 9 L glioma cells was monitored by MRI and optical imaging in vivo. They were also able to follow the silencing process by optical imaging. With this probe, Medarova et al. demonstrated a novel non-invasive approach to siRNA delivery and monitoring using nanoparticles.

Multifunctional/multimodal nanoparticles

Multifunctional or multimodal nanoparticles (MMNPs) are nanoparticles that combine several different functional capabilities in a single stable unit. For example, a core nanoparticle could be linked to a specific targeting ligand that recognizes the unique surface signature on their target cells. Simultaneously, the same particle can be attached with an imaging agent to monitor its transport progress, a moiety to evaluate the therapeutic efficacy of a drug or a therapeutic agent. In short, the function of the multifunctional nanoparticle depends on the component attached. With multiple components such as fluorescent molecules, tumor targeting moieties, anticancer drugs, or siRNA available the possibilities are numerous [52].

MMNPs in bioimaging and biosensing are a rapidly growing research field as these particles can be detected using multiple imaging modalities, i.e., MRI, X-ray, ultrasound and fluorescence, unlike their single-modal predecessors. The use of MMNPs as contrast agents will enable intra-operative imaging as well as improve pre-op imaging, offering new approaches to visualization and accurate resection of tumors.

Silica based nanoparticles have been developed for the purposes of bioimaging and biosensing [53]. Silica is a good candidate for this work because it is a good host material for agents such as fluorescent dyes, metal ions, and drugs. The silica matrix is transparent and allows excitation and emission light to pass through efficiently. Encapsulation by silica also provides enhanced photostability [53] for optical agents. The surface of silica particles can be modified easily to attach bio molecules; it is water dispersible, resistant to microbes, biocompatible and resistant to swelling due to changes in solvent polarity. These characteristics of silica have led to its use in the production of several MMNPs. In 2007, Lu et al. used SPIO nanoparticles coated with silica conjugated to fluorescein isothiocyanate to label human bone marrow mesenchymal stem cells [54]. In 2006, Lee et al. demonstrated the use of dual-mode nanoparticles for imaging of neuroblastoma with MR and fluorescence imaging [55]. They fabricated a “core satellite” structure, comprised of a rhodamine dye-doped silica “core” and multiple “satellites” consisting of magnetic nanoparticles. These hybrid nano-

particles were then conjugated with HmenB1 antibodies, known to specifically target cells expressing polysialic acids. Polysialic acid is a tumor marker for neuroblastoma, lung carcinoma and Wilms tumor. Using optical and MR imaging the targeted cells were visualized and the results were found to be consistent with polysialic acid expression at the cell membrane [55].

Liong et al. demonstrated a nanoparticle consisting of SPIO nanocrystals incorporated in mesoporous silica nanoparticles with attached hydrophilic groups, which allowed the monitoring of living cells via MR and fluorescence imaging [52]. As the mesoporous silicate had targeting ligands and chemotherapeutic drug molecules attached to it, the particle was also capable of simultaneous imaging and therapy as well [52]. This exemplifies the concept of “nanoclinics”, multiple function nanoparticles that detect, treat and monitor cancer. Again as with the previous nanoparticles, these imaging agents will be able to target specific cells and induce therapy on these particular cells, collateral damage to normal neighboring cells will be significantly reduced.

Promising in vitro studies have led to preclinical evaluation of nanoparticles for imaging cancer. Several promising nanoparticles are emerging for imaging important cancers including breast, prostate, brain and pancreatic. Joshi et al. developed a handheld magnetometer which they successfully used to detect sentinel nodes in a series of breast cancer patients, using an SPIONs injected subcutaneously [56].

Imaging applications in common cancers (Table 2)

Breast cancer

In England, over 44,000 women are diagnosed with breast cancer each year [57]. Therefore it is not surprising that there are a significant number of studies done in this area to identify novel uses for several different nanoparticles in the diagnosis and treatment of breast cancer. In 2004, Kim et al. [58] successfully demonstrated type II NIR (near infrared) QDs for the mapping of sentinel lymph nodes in both large and small animals.

This method eliminated the need for both a radioactive tracer and a blue dye as use lymph flow and the SLN was identified optically. It could also be used by the pathologist to identify the nodes after resection [58]. This study did not look into the toxicity of QDs. In 2007, Lee et al. [59] carried out studies to determine if magnetism-engineered iron oxide (MEIO) nanoparticles conjugated with cancer targeting antibody, trastuzumab, could be used to detect cancer on MR imaging. Trastuzumab specifically binds to HER2, a marker which is overexpressed in breast and ovarian cancer. In vivo results indicated that the high MR

sensitivity of MEIO-trastuzumab conjugates enabled the MR detection of very small tumors (about 50 mg).

SPIONs are under investigation as an alternative method of mapping the sentinel lymph node (SLN) in breast cancer. The current gold standard for sentinel lymph node biopsy is the “combined technique”. This involves the use of blue dye as well as radioisotopes in order to detect the node visually intra-operatively and by also using a gamma probe. This technique has several drawbacks; the blue dye obscures the surgical field and may cause tattooing of the skin. The radioisotope used exposes the patient and medical staff to the hazards of radiation. Its use is heavily regulated by legislation and specialized training and disposal methods are required. As SPIONs provide better preoperative images on MRI when compared to radioisotopes and do not have the drawbacks stated above, they provide an attractive alternative to radioisotopes for mapping sentinel lymph nodes. Joshi et al. [56] have developed a handheld magnetometer, the SentiMag, which is capable of detecting the SPION contrast in the lymph nodes once it has been injected subcutaneously into the affected breast (Fig. 2). A pilot study on 10 patients demonstrated that the SPION/SentiMag technique is as reliable as the “combined technique”. A larger study is underway.

Several nanoprobe have been designed using trastuzumab. These particles can potentially be used to carry out treatment, by delivering therapeutic agents or causing thermal ablation of the targeted cells. Bagalkot et al. designed a nanoparticle composed of a QD, an aptamer for cell-specific targeting and a molecule of doxorubicin [60]. This combination enabled this multifunctional particle to perform in vitro targeted imaging and therapy as well as sensing drug release [60]. In vitro studies carried out on breast carcinoma cells using nanoshells have shown that they are able to carry out targeted photothermal destruction of cancer cells when exposed to near infra red light. The cell damage was found to be limited to the area treated by laser, thus confirming reduced collateral damage and target cell specificity. Further in vivo studies have also been carried out [13].

Recently Wang et al. [61] demonstrated a novel route to synthesize a multifunctional nanoparticle-pearl-necklace based on Fe_3O_4 (magnetite) nanoparticles decorated with gold nanorods (Au_{rod}). Comparing Fe_3O_4 and $\text{Au}_{\text{rod}}(\text{Fe}_3\text{O}_4)$ as MR contrast agents; they found $\text{Au}_{\text{rod}}(\text{Fe}_3\text{O}_4)$ had a stronger magnetization than the bare Fe_3O_4 particles. These particles can also be used as a fluorescence-imaging agent to target cancer cells [61]. In order to target breast cancer cells (SK-BR-3) these nanoparticles with conjugated with trastuzumab. A significantly increased rate of internalization was seen in $\text{Au}_{\text{rod}}(\text{Fe}_3\text{O}_4)$ particles that were conjugated with trastuzumab compared to bare $\text{Au}_{\text{rod}}(\text{Fe}_3\text{O}_4)$ nanoparticles. The findings proved that trastuzumab-mediated endocytosis of PEGylated $\text{Au}_{\text{rod}}(\text{Fe}_3\text{O}_4)$ n-trastuzumab

Table 2 Nanoparticle applications in common cancers

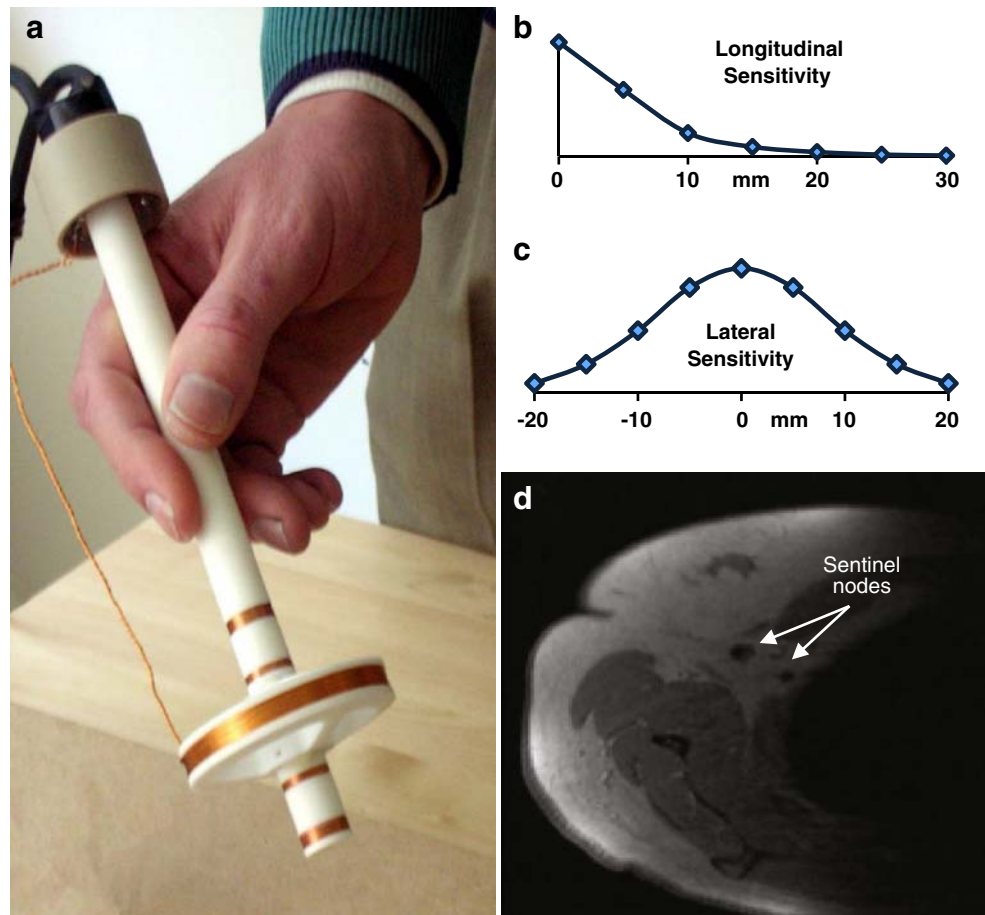
Cancer site	Nanoparticle	Clinical application	Current status
Breast	Iron oxide nanoparticles + Herceptin	Detection of small tumors on MRI	Preclinical
	Iron oxide nanoparticles + uMUC-tumor antigen	MRI and monitoring tumor response to chemotherapy via antigen expression and change in size	Preclinical
	Dendrimer	Contrast agent for micro-MR lymphangiography	Preclinical
Colon	Iron oxide nanoparticle	Detection of sentinel lymph node	Clinical
	Iron oxide particles	MRI of CRC and metastases	N/A
Prostate	QDs	Visualization of cancer using fiber optics	Preclinical
	Iron oxide nanoparticles	Detection of metastasis with high resolution MRI	Preclinical
Brain	Dendrimer + Prostate specific antibody	Targeting of antigen expressing cells	Preclinical
	Iron oxide nanoparticles	Dual function particles to help define tumor margins accurately intra-operatively	Preclinical
Pancreas	Iron oxide particles	Enhances normal pancreatic tissue on MRI enabling easy visualization of PDAC	Preclinical

nanoprobes can be used for the targeting and labeling of breast cancer cells [61].

Medarova et al. [62] have recently designed a particle capable of monitoring a patient's response to chemotherapy with MRI. It is not only tumor size that is monitored but also target antigen expression. The particle consists of SPION

particles modified with Cy5.5 attached on a peptide (EPPT), which specifically recognizes uMUC-1. Underglycosylated MUC-1 (uMUC-1) is a tumor antigen found in 90% of breast cancers and is predictive of chemotherapeutic response [62]. In vivo treatment of mice bearing human breast carcinoma with doxorubicin led to a reduction in tumor mass as well as

Fig. 2 Handheld SPION-detecting magnetometer trial. **a** Handheld magnetometer for the detection of SPION particles (the SentiMag probe made by Endomagnetics Ltd, London UK). **b** & **c** Readings from laboratory work showing detection of the SPIONs particles by the SentiMag. **d** Sentinel lymph node enhanced on MRI with SPION contrast agent “Endorem”



uMUC-1 expression. As this method involves monitoring of antigen expression as well as tumor size it is a more sensitive measure of tumor response to treatment.

Colorectal cancer

Bowel cancer is the third most common cancer in the UK, with 36,500 people diagnosed with bowel cancer each year, or the equivalent to a 100 people every day [57]. Diagnosis still relies on symptoms although screening with colonoscopy has recently been introduced in selected patients. Imaging-based detection needs to be improved in order to develop targeted drug delivery and techniques for ablation of metastases. Iron oxide nanoparticles or iron nanoshells may prove to be a suitable contrast agent for MR imaging of colorectal cancer. Guanyl cyclise C (GCC), has been presented as a suitable receptor to target colorectal cancer micrometastases [63]. It may be possible to use GCC as a targeting ligand by conjugating it on to multifunctional nanoparticles. Targeted imaging and treatment of colorectal cancer can then be carried out. As fiber optics are currently used in the investigation of colorectal cancer, i.e., endoscopy, near infrared (NIR) imaging can be easily introduced into the clinic [64]. It is possible to enhance endoscopic visualization of colorectal cancer by using tunable QDs. A murine model of colorectal cancer studied for this purpose showed promising results [65].

Prostate cancer

In 2005, 34,302 new cases of prostate cancer were reported in the UK, amounting to 24% of cancers in the men [57]. In 2003, Harisinghani et al. demonstrated that SPION, could be used to detect small otherwise undetectable lymph node metastases in patients with prostate cancer using high resolution MRI [20]. The nodes detected on high resolution MRI were verified using histological studies. Using a fluorescein-tagged dendrimer, which was conjugated to a prostate specific membrane antibody, Thomas et al. [66] demonstrated the ability to target and image antigen expressing cells. Flow cytometry, confocal microscopy, and a two-photon-based optical fiber fluorescence detection technique were used to cross verify that the conjugates bound specifically to antigen-expressing cells. This study demonstrates that dendrimer-antibody conjugates can be used to target antigen-expressing cells with accuracy.

Brain cancers

In 2005, 4,555 people in the UK were diagnosed with brain and other central nervous system tumors [57]. Gliomas are the commonest and most lethal type of primary brain tumor. Accurate evaluation of tumor volume using modern imaging

is difficult due to the presence of oedema around the tumor site. In addition, contrast delivery to the brain is hindered by the blood brain barrier (BBB). The BBB is a specialized system of endothelial cells that separates the general circulation from the brain, providing protection to the cells of the brain and preserving homeostasis [9]. Nanoparticles coated in surfactant (polysorbate) or conjugated with peptides are able to cross the BBB. The exact mechanism of nanoparticle transport across the BBB is still unknown. It is believed that polysorbate-coated nanoparticles mimic LDLs and are transported into the brain by endocytosis [67]. The ability of nanoparticles to reach the brain will significantly increase the quality of brain tumor imaging.

Surgery is important in the treatment of brain cancer. Distinguishing between cancerous cells and normal brain tissue visually intra-operatively is still challenging. Accurate resection is paramount in order to achieve complete resection and limit morbidity. Dual imaging nanoparticles, detectable by both MRI and fluorescence microscopy may be able to overcome this limitation as demonstrated by the work done on dendrimers by Shi et al. [42]. The functionalized Au DENPs demonstrated by them could potentially be used for pre-surgical planning (MRI) and also intra-operatively, during resection (optical imaging). Veisheh et al. [67] also demonstrated that a nanoprobe, which was preferentially taken up by glioma cells, enabled MR and fluorescence imaging of the tumor. The probe consisted of an iron oxide particle coated with PEG, which was conjugated with chlorotoxin, a unique peptide shown to bind specifically to glioma, and the near infrared fluorescing molecule Cy5.5. In addition to its preferential targeting abilities, the probe was found to be highly stable and have a retention time of about 24 h in the targeted cells. It is hoped these particles could be used pre- and post-operatively for diagnosis and monitoring of the tumor and also intra-operatively for visualization of tumor margins enabling more effective treatment of brain tumors than available at present [67].

Pancreatic cancer

Pancreatic cancer is the tenth most common cancer in the UK, with an average of just over 20 cases diagnosed every day [57]. Studies have looked at identifying a targeting agent that could help to characterize pancreatic tissue. Bombesin (BN), a peptide isolated from the European frog *Bombina orientalis* [68], was found to bind to healthy pancreatic tissue in earlier studies. Montet et al. used this to come up with an inverse strategy to image pancreatic ductal adenocarcinoma cells (PDAC). They designed a nanoparticle conjugate based on a cross-linked iron oxide (CLIO) particle, to target bombesin receptors present on normal acinar cells of the pancreas. The BN-CLIO nano-

particle decreased the intensity of normal pancreatic cells and thus enhanced the ability to visualize PDAC on MRI, offering a promising new approach to imaging PDAC [68]. Distinguishing malignant from benign pancreatic cells, on imaging, is still a challenge.

Discussion and conclusion

Nanotechnology has been described as the “small technology with a big impact.” Considering the many possibilities it has opened up in the fields of engineering, materials science and electronics it is clear that nanotechnology is here to stay. Nanoparticles applied to medicine are all biocompatible with the exception on QDs. By coating these nanoparticles with stealth polymers such as PEG their biocompatibility and circulation times can be increased [69]. Nanoparticles display the ability to concentrate preferentially at tumor sites due to the EPR effect. This effect can be enhanced by attaching targeting ligands. It is possible to attach a variety of moieties on to their surfaces in order to design particles to suite particular applications. Most of the particles have been used as a constituent of a dual or multifunctional probe to demonstrate combined imaging and treatment techniques. This ability to simultaneously image and treat tumors with minimal side effects is a clear advantage over contrast imaging techniques.

Surgery is still the most important treatment for most solid cancers. Excision is the ultimate cure for cancer. But surgery has its limitations. The inability to distinguish between cancerous cells and normal tissue visually intra-operatively is an important clinical challenge. Dual modality nanoparticles combines high spatial resolution, tomographic capability, and the unlimited tissue penetration of MRI, with the high sensitivity and low cost of in vivo optical imaging [70]. Dual imaging nanoparticles, detectable by both MRI and fluorescence, could help to overcome this limitation and improve the accuracy of surgery.

Another important clinical challenge in the management of cancer is the inability to detect micro-metastases in vivo. Adjuvant chemotherapy is often administered on the basis of risk of metastasis, as a result of this. The ability to use nanoparticles, i.e., QDs to follow cancer cells as they metastasize will lead to a greater understanding of the metastatic cascade and may lead to the discovery of new modes of treatment.

Nanotechnology has blurred the boundary between diagnosis and treatment. Traditionally, diagnosis and treatment were considered as two separate entities in the process of patient care. With the emergence of multifunctional nanoparticles, these two distinct clinical processes will soon merge into one. It may become possible to treat patients at the same time as diagnosis, thus alleviating the

agonizing wait between undergoing diagnostic tests, results and receiving definitive treatment. This could have a significant impact on current clinical practice. This also raises ethical issues. In some situations patients will have to be informed and give consent for possible treatment before the diagnostic tests are carried out, in case treatment can be carried out simultaneously. Communication skills will be challenged, as patients may have to be advised about possible treatment options before a definitive diagnosis has been reached. Better quality pre-operative MRI could improve pre-operative planning further and targeted treatments could reduce unwanted side effects and improve the quality of life.

The possibility of personalized medicine, targeting a specific patient group, has been discussed in the last few years. Trastuzumab for breast cancer is a good example. This relies on good bench to bedside and bedside to bench research. Nanomedicine needs strong clinical support in order to identify clinical challenges, select the most promising nanoparticle and plan clinical trials. The latter is lacking at present. Future effort should focus on more translational research that focuses on clinical application.

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