A NEW GENERATION OF NANOPARTICLES FOR IMAGE ENHANCEMENT

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Introduction

Noninvasive and minimally invasive *in vivo* imaging techniques allow for the facile acquisition of internal image data, negating the need for surgical procedures, saving time and cost whilst providing minimal discomfort to patients. Noninvasive imaging techniques are primarily employed to detect and locate cancer (1), cardiovascular diseases (2) and anatomical problems. A variety of imaging techniques have been developed in recent years (each with strengths and weaknesses, see **Table 1**), including magnetic resonance imaging (MRI), computer tomography (CT), positron emission tomography (PET) and fluorescence optical imaging. The process used for selecting the appropriate imaging technique for initial diagnosis is usually guided by the type of disease (and cost). Imaging techniques often require an extraneous imaging agent to be administered to the patient to enhance the imaging signals (enhancement of sensitivity, spatial or temporal resolution, cost, etc), leading to the commercial development of a large range of small organic contrast agents. More than one third of MRI scans are performed in conjunction with a contrast imaging agent. The first generation of organic contrast agents had some limitations (poor specificity and rapid renal clearance) (3,4). More recently, contrast agents have been designed in a nanoparticle form (5). Nanoparticles are a class of materials with a size in the range of nanometres (10^{-9} m), typically with a size from 1 to 100 nm (about 10^4 times smaller than cellular dimensions). The incorporation of contrast agents into nanoparticles can offer significant advantages, such as easy functionalisation by targeting moieties and improved biodistribution. In this mini-review, we will highlight the use of new nanoparticle imaging agents in modern bioimaging methodologies, which holds great promise for significant improvements in imaging technology in the near future.

Magnetic Resonance Imaging (MRI)

MRI is a widely used imaging technique commonly employed at large public hospitals. MRI yields tridimensional images with good resolution but limited sensitivity. To improve sensitivity and specificity, a contrast agent is often administered to the patient (**Table 2**). Different nuclei can be detected using MRI, including hydrogen and fluorine. However, only hydrogen MRI is employed routinely in clinics. Over 33% of the hydrogen MRI scans taken clinically are performed in conjunction with contrast agents, such as gadolinium (Gd) containing compounds to enhance the signal derived from hydrogen atoms (present as water within tissues). Gadolinium-based contrast agents are widely employed for MRI, however, in a small minority of cases, complications can occur (nephrogenic systemic fibrosis) and the FDA has recommended restricting the use of Gd contrast agents where patients exhibit kidney deficiency (6). In use, Gd is placed in a molecular cage (or complex) to reduce toxicity and enhance solubility *in vivo*. However, at high magnetic fields, the complexed Gd contrast agents lose their MRI enhancing effect (i.e., relaxivity) resulting in decreased sensitivity. To enhance the relaxivity of Gd contrast agents, several groups have investigated the attachment of Gd atoms into larger nanostructures – this restricts the mobility of the Gd atoms, which in turn increases their relaxivity (providing enhanced MRI images) (7). There is no doubt that nanoparticle-attached Gd can significantly enhance MRI signals, but presently, concerns still need to be addressed about bioaccumulation and stability of the nanoparticle formulations *in vivo*. Consequently, researchers have been looking into alternative (less toxic) contrast agents, and iron oxide nanoparticles (8) have also been investigated as negative contrast agents in MRI. Iron oxide nanoparticles have proved to be non-toxic both

Table 1. Characteristics and performance of common imaging techniques.

Imaging modality	Resolution *(in vivo)*	Signal measured	Depth	Sensitivity	Cost	Imaging time
Proton MRI	25–100 µm	Alteration of magnetic field	No limit	10^{-3}–10^{-5} M; 10^{-6}–10^{-9} M for contrast agents	Very high	Minutes to hours
Computer tomography	20–50 µm	X-rays	No limit	10^{-2}–10^{-3} M for organic contrast agent; 10^{-10} M for nanoparticles	High	Minutes
Fluorescence imaging	1–3 mm	Light, usually near infra-red	< 1 cm	10^{-12} M	Low	Seconds to minutes
PET	1–2 mm	Positron from radionuclides	No limit	10^{-10}–10^{-12} M	Very high	Minutes

Table 2. Advantages and disadvantages of different imaging techniques.

Imaging modality	Probe	Advantages	Limitations
Proton MRI	Paramagnetic metals (e.g., Gd and Mn); super-paramagnetic nanoparticles (e.g., iron oxide); hyperpolarised probes (^{129}Xe)	High spatial resolution, very high soft tissue discrimination, combined anatomic and functional imaging	Low sensitivity, long scan, high mass of probe, possible toxicity side effect for Gd
Computer tomography	10^{-2}–10^{-3} M for organic contrast agent; 10^{-10} M for nanoparticles	High spatial resolution, excellent bone imaging	Radiation, limited soft-tissue discrimination, high concentration of molecular contrast agent
Fluorescence imaging	Quantum dots, dye-doped nanoparticles, carbon-based nanomaterials	High sensitivity, multiplexed imaging, immense catalogue of probes	Poor depth penetration, relatively low spatial resolution
PET	Nanoparticles incorporating radioisotopes (e.g., ^{18}F, ^{11}C, ^{64}Cu, ^{124}I)	Very high sensitivity, quantitative, very low concentration required	Radiation, relatively low spatial resolution, need cyclotron or radioelement source

in vitro and *in vivo*, and can exhibit a high transversal relaxivity (r_2) yielding a negative contrast in MRI images. However, iron oxide nanoparticles are unstable in biological media and require stabilisation (often achieved using a polymer coating) (8). A number of stabilising polymer layers have been described in the patent and journal literature: natural (dextran) and synthetic (poly(ethylene glycol); PEG) polymers have been used. PEG has been demonstrated to stabilise iron oxide nanoparticles for a long period in biological media, while dextran-coated nanoparticles tend to accumulate in the liver (9). Several different linkers have been used to anchor polymers to iron oxide nanoparticle surfaces, e.g., silane, phosphonic acid, and carboxylic acid (10).

Fluorescent Nanoparticles for Optical Imaging

Optical imaging in conjunction with nanoparticles incorporating fluorescent dyes or quantum dots can facilitate imaging *in vitro* or *in vivo* (in small animals) without the requirement for sophisticated or expensive instruments. The imaging performance of dyes can be enhanced by encapsulation in polymeric nanoparticles, improving contrast and boosting signal:noise ratios (this favourable result comes from a significant local dye concentration enhancement in the nanoparticle structure). However, it is important to optimise the nanoparticle design to avoid quenching effects. To allow for signal detection through the skin (and other tissues), dyes with an excitation and emission wavelength range of 600 nm to 900 nm are employed, i.e., in the near infra-red region (11).

Quantum dots, based on semi-conductors, have also been employed for optical imaging in recent years as they exhibit high quantum yields and good *in vivo* stability (resulting in less photo-bleaching) (12). However, quantum dot use *in vivo* is limited by their potential toxicity as they are made using cadmium (some recent evidence seems to indicate that cadmium can, in some cases, be used safely) (13). On excitation, the quantum dot can sometimes release toxic cadmium via photolysis (14,15) – a clearly undesirable outcome. To avoid the release of toxic metal, the quantum dot can be coated with polymers, improving colloidal stability. Polymer coating is generally perceived as desirable in minimising any potential toxicity. Silicon quantum dots have recently been developed (15), with sizes in the range of 1 to 6 nm (16). Silicon quantum dots can also be functionalised with polymer coatings to enhance stability whilst providing a scaffold for targeting.

Computer Tomography (CT)

Computer tomography is an X-ray imaging technique employed to visualise hard tissues, such as bone. Contrast enhancement is often achieved using iodine compounds (primarily) (17) or gold nanoparticles (18) for soft tissue. The high atomic number of iodine creates high contrast. Polymer-stabilised gold nanoparticles, with a size ranging from 2 to 10 nm, have been investigated as potential contrast agents (19). Thiol, disulfide or other sulfur derivative (dithioester and trithiocarbonate) terminated polymers or organic compounds can be easily attached to gold surfaces, providing an anchor for polymer coatings (or brushes). Thiol-gold interactions are relatively weak (unstable at high temperatures or in the presence of other thiols). However, in the case of polymer grafted onto gold nanoparticles, thiol displacement is significantly slowed by steric hindrance. Cross-linking the polymer layers has also been proposed to improve the stability (20). Gold nanocage nanoparticles have also been employed as dual contrast agents for combined optical and PET imaging of tumours (21,22), as it is possible to tune the gold plasmon resonance band by adjusting the size of the nanocages from 600 to 1100 nm (23).

Positron Emission Tomography (PET)

Positron emission tomography employs radioelements that emit gamma radiation and requires very small concentrations (10^{-9} M) of emitter. Different radioelements are commonly employed, each with a characteristic half-life: ^{11}carbon, ^{15}oxygen, ^{18}fluorine, ^{64}copper, etc. The most commonly employed elements are fluorine and copper, as their half-lives are most suitable for clinical usage. Fluorodeoxyglucose (FDG, a glucose derivative) is widely used in the clinic to detect tumours, as cancer cells require a large amount of sugar for their development (enhancing

uptake). Radioelements have also been encapsulated into polymeric nanoparticles, as shown by Wooley, Hawker and co-workers (24,25), who encapsulated ^{64}copper into polymeric micelles, using DOTA (a polydentate ligand).

Bio-distribution of Nanoparticles

Nanoparticle design parameters (size, polymer length and shape) can be tuned to optimise bio-circulation. Nanoparticles in the range of 10 to 100 nm are optimal for a long circulation time *in vivo*. Large particles (> 200 nm) are readily sequestered by the phagocytic cells in the spleen (26) or by the macrophage cells present in blood, while very small sized nanoparticles (< 10 nm) are rapidly removed by renal clearance (27). For bio-imaging applications, the nanoparticle size must be tuned according to the required clinical criteria. For instance, when toxic elements such as Gd or cadmium are used, it is desirable to have short circulation times (just long enough for the imaging analysis) followed by rapid excretion. A number of nanoparticle design guidelines have been established in the course of a few studies. Perrault and co-workers examined the effects of core size (hard part) and the chain length of polymers (PEG) (soft part) on the bio-distribution of iron oxide nanoparticles, finding that blood circulation half-life decreased as the core diameter increased; conversely, an increase of PEG molecular weight was found to cause a significant increase in circulation time (28). Shape is another factor in determining the stability and biodistribution of nanoparticles (39,30). Theoretical models have been used to study the effect of nanoparticle shape on blood circulation times. Decuzzi and Ferrari established, via simulation, that oblate spheroid nanoparticles will circulate longer than comparable spherical nanoparticles (31). Recently, this theoretical result has been validated by several experimental studies. For instance, Muro *et al.* compared the biostability of various diameter spheres versus elliptical discs, showing that elliptical discs have longer circulation times (32). A similar result has been reported for spherical gold nanoparticles and nanorods, where cell uptake was found to be three times higher for spherical gold nanoparticles (33). In conclusion, nanoparticle shape is a significant factor affecting bio-distribution. Spherical particles need to have sizes smaller than 200 nm to pass through the spleen; in contrast, elliptical nanoparticles (discs) with sizes greater than 1000 nm can pass through, a result explained by auto-organisation (alignment or tumbling) of non-spherical nanoparticles under the influence of flow (31).

Long nanoparticle (sizes inferior to 500 nm) blood circulation times are known to be beneficial in promoting accumulation in tumours and at inflammatory and infection sites due to the enhanced permeability and retention effect (EPR). The EPR effect is caused by an increased permeability of the vascular system and inefficient lymphatic drainage close to tumour sites. Passive targeting (EPR) has been demonstrated for nanoparticles with sizes ranging from 10 to 500 nm (34). Perrault *et al.* studied the effect of nanoparticle size (ranging from 20 to 100 nm) on accumulation at tumour sites, finding that particle accumulation (sizes from 40 to 100 nm) depends only on the blood residence half-life, independent of nanoparticle size; in contrast, for smaller particles (sizes around 20 nm) the accumulation depends on both (28). However, small particles have a relatively short residence time at the tumour site when compared to larger particles (> 40 nm). In summary, small nanoparticles arrive rapidly at the tumour site, but they also have a short residence time. In contrast, larger nanoparticles take longer to reach the tumour sites, but reside for longer. The residence time is a key parameter for therapeutic efficiency, as nanoparticles are initially transported in blood, followed by passive diffusion from the blood vessel to the tumour periphery. The distribution (and therefore efficiency) of the nanoparticles depends on both their size and their residence time in the vicinity of the tumour (28).

A different passive targeting technique has been developed for iron oxide nanoparticles using reticuloendothelial system (RES) clearance to facilitate imaging of specific organs, such as the spleen or liver. RES clearance targeting relies on a rapid uptake of dextran-coated nanoparticles by macrophages. The first commercial contrast agents (Feridex IV; based on iron oxide nanoparticles) exploited this mode of targeting to image infected tissue in the liver (35). The rapid uptake of iron oxide nanoparticles by Kupffer cells in the liver facilitated differentiation of healthy tissues from tumour cells using MRI. However, macrophage-assisted targeting is limited to specific organs (liver and spleen, both rich in macrophages) or inflamed tissues.

Improved imaging resolution can also be envisaged by adopting an active targeting strategy using biological recognition events. Different targeting compounds have been described in the literature, namely, peptides (RGD, NGR) (10), proteins (monoclonal antibodies) (36,37), aptamers (38,39), carbohydrates (40) and small molecules (folic acid) (41). For example, Wang *et al.* modified iron oxide nanopartilces with a poly(amidoamine) dendrimer, followed by folic acid attachment, enhancing uptake by KB-HFAR cells (a human epithelial carcinoma cell line that expresses high levels of folic acid receptor) (42). An active-targeting approach has the potential to reduce the contrast agent concentration required for clinical use whilst still maintaining sufficient resolution. The attachment of targeting functionality to nanoparticles can be achieved using a number of synthetic strategies, including carboxylic acid-amine reactions (43), click chemistry (i.e., copper-catalysed azide–alkyne cycloaddition, CuAAC) (40,44), pyridyl disulfide-thiol exchange and thiol-ene reactions (45,46). The accessibility of targeting groups on nanoparticle surfaces is important, as shown by Martin *et al.*, who found that accessibility induced different biological responses (40). Targeting moieties (i.e., lactose) fixed on a dendrimer enhanced exposure and binding (by 1–2 orders of magnitude) over monofunctional lactose terminated polymers. An enhanced availability of the dendritic molecules on the surface prevented interment of the lactose within the polymer coating. In addition, the fixed presentation of the targeting moieties by the dendritic structure enhanced binding via a concentration effect (known as multivalency). The study by Martin *et al.* proved that the binding affinity of carbohydrate ligands on polymer surfaces could be significantly enhanced

using dendritic scaffolds. However, the importance of multivalent interactions depends to some extent on the specific biological binding event and therefore some caution should be exercised in extending the conclusions from the study of Martin *et al.* to all targeting interactions.

Nanoparticles for Dual Imaging and Drug Delivery

The possibility to track and carry therapeutic molecules to specific sites, using multi-modal nanoparticles, has inspired a large number of recent studies, for example, Labhasetwar and coworkers (47) encapsulated doxorubicin in iron oxide nanoparticles. Drug release can be engineered by exploiting different stimuli, e.g., pH, enzymes or redox responses (48). We recently engineered hybrid polymer/iron oxide nanoparticles to deliver small interfering ribonucleic acid (siRNA) that could be tracked by MRI (in collaboration with the Children's Cancer Institute Australia) (10,49). Combined imaging and drug release from nanoparticles has been described in a wide range of studies, including PET imaging and doxorubicin delivery (50).

Conclusion

The design of sophisticated nanoparticles for enhancing bio-imaging is a fast growing field. The potential ability to image (track) and deliver therapeutic molecules simultaneously has excited a lot of interest and the skills required for progress are truly multidisciplinary, requiring inputs from nanotechnologists, polymer chemists, clinicians and physical scientists.

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