NANOPARTICLES FOR DIAGNOSTIC IMAGING AND RADIOTHERAPY SPECIAL FEATURE: REVIEW ARTICLE

Nuclear molecular imaging with nanoparticles: radiochemistry, applications and translation

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ABSTRACT

Molecular imaging provides considerable insight into biological processes for greater understanding of health and disease. Numerous advances in medical physics, chemistry and biology have driven the growth of this field in the past two decades. With exquisite sensitivity, depth of detection and potential for theranostics, radioactive imaging approaches have played a major role in the emergence of molecular imaging. At the same time, developments in materials science, characterization and synthesis have led to explosive progress in the nanoparticle (NP) sciences. NPs are generally defined as particles with a diameter in the nanometre size range. Unique physical, chemical and biological properties arise at this scale, stimulating interest for applications as diverse as energy production and storage, chemical catalysis and electronics. In biomedicine, NPs have generated perhaps the greatest attention. These materials directly interface with life at the subcellular scale of nucleic acids, membranes and proteins. In this review, we will detail the advances made in combining radioactive imaging and NPs. First, we provide an overview of the NP platforms and their properties. This is followed by a look at methods for radiolabelling NPs with gamma-emitting radionuclides for use in single photon emission CT and planar scintigraphy. Next, utilization of positron-emitting radionuclides for positron emission tomography is considered. Finally, recent advances for multimodal nuclear imaging with NPs and efforts for clinical translation and ongoing trials are discussed.

OVERVIEW OF NANOPARTICLES FOR IMAGING—ROLE OF PHYSICOCHEMICAL PROPERTIES AND BIOLOGICAL CHARACTERISTICS

For applications in biomedical imaging, desirable nanoparticle (NP) properties include robust imaging signal for a given modality, biocompatibility (and perhaps biodegradability) and versatility of surface chemistries, which can enable conjugation of ligands and/or bioactive structures. Generally, these requirements hold across modalities, including MR, optical, ultrasound and radioimaging. The chemical versatility of NPs enables multiple features to be available in a single agent. Functionality in the form of stealth-like coatings (to enable long circulation), affinity ligands (to enable targeted uptake), biodegradable constituents (to enable drug release and biocompatibility) and imaging or radiological detection (to enable long or short sensitive tracking and identification in vivo) is of great value. The biological fate of NPs can be modulated through primary design considerations and post-synthesis modification; particularly with respect to size, charge and hydrophilicity/phobicity characteristics. This is largely in contrast to small molecule radiotracers, which make up the bulk of probes used in nuclear medicine. NPs can effectively act as platforms allowing for a high degree of tailored modification for precise properties of interest. Additionally, NP radiosynthesis can be achieved with excellent radiolabelling efficiency and rapid synthesis and purification, which are often required for nuclear imaging applications utilizing short-lived radioisotopes.

For imaging applications, probe distribution is a key parameter. Pharmacokinetically, NP blood residency time and tissue uptake are predominantly dependent on size, surface chemistry and charge. While targeting bioactive entities (such as conjugated peptides or conjugated antibodies) may enhance uptake to sites of disease, the hydrodynamic diameter critically determines residency time and clearance.
routes. Parenterally administered, agents below the glomerular filtration cutoff (30–50 kDa or a diameter of approximately 5 nm) are rapidly excreted by the kidneys. Larger NPs may circulate longer, but often accumulate in the liver and spleen, while micron-sized particles become entrapped in capillary beds and the pulmonary vasculature. In response, the surfaces of the NPs are commonly modified with a polysaccharide or polymer, coatings which decrease recognition by passive and active clearance mechanisms, increase blood residency, circulation time and achieve greater uptake at sites of interest.

Furthermore, highly charged and hydrophobic particles are rapidly opsonized by serum proteins, a process that increases diameter and enhances recognition by the reticuloendothelial system (RES).2 Below 100 nm in diameter, particles may circulate for longer, enabling sites of disease to be visualized through the enhanced permeability and retention effect (EPR).3 This phenomenon results in non-specific accumulation of macromolecules and NPs at sites of compromised vasculature and blood flow—such as sites of malignancy. Beyond mere size, shape and aspect ratio (length:width) are also determinants of clearance route and kinetics.1

Nanomaterials for biomedical imaging

A diversity of nanomaterials has been used for the purpose of nuclear imaging. These include lipids, proteins, carbon-based and metal-based particles. The following sections will briefly overview particle classes and their properties.

**Liposomes**

Liposomes were first synthesized by Bangham4 and are perhaps the longest studied NPs used for biomedical applications. These lipid structures are amphiphilic spherical vesicles, comprised of a phospholipid (PPL) bilayer or series of bilayers, and whose diameter may be tuned from 80 nm (unilamellar) to 1000 nm (multilamellar). Both hydrophilic and hydrophobic materials can be encapsulated within liposomes. Polyethylene glycol (PEG) polymer coatings limit clearance and extend the circulation time in the blood by shielding the negative charges of the PPL heads.5 Conversely, the same negative charges create a niche for electrostatic interactions, trapping positively charged isotopes or small molecules at the NP surface (Figure 1a–c).6

**Quantum, silica and Cornell dots**

Quantum dots (QDs) are fluorescent nanocrystals (1–10 nm diameter) made of a combination of semi-conductor metals including: CdSe, CdS, CdTe, ZnSe, InP, InAs, PbSe (Figure 1d).7,8 QDs have unique optical and electrical properties, such as bleach-free fluorescence and size-tuned emission characteristics. Composition, coating and size are determining factors of QDs emission wavelengths. CdSe, CdTe, InP, PbS and PbSe particles emit in the range of 470–660, 520–750, 620–720, 900 and 1000 nm, respectively. CdSe NPs emit at different wavelengths in a size-dependent manner: the smaller the particle, the shorter the emission wavelength.9 Regarding blood circulation time, these small particles can be rapidly excreted.10 Bare QDs are not always stable in aqueous or biological environments and can be coated by hydrophilic polymer layers such as PEG11 or short-length peptides12 for water-solubility, however, the increased NP size (>5 nm) sees a shift from renal to hepatic clearance.

Concerns about heavy metal toxicity of QDs hamper widespread use. More biocompatible particles have been developed using carbon1 and also silica.13 The latter has been used in Cornell dots (C-dots), which are ultrasmall particles (3–5 nm) consisting of a fluorophore encapsulated by a biodegradable shell of...
silica. In contrast to QDs, which when PEG coated are re-routed to the liver, C-dots are still rapidly excreted via glomerular filtration. Thus, the in vivo residency time is very short. This has potential advantages for radioactive dosimetry and long-term toxicity.

Carbon-based nanoparticles
Carbon nanotubes (CNTs) and graphene are both carbon-based materials with well-defined physical and chemical properties. CNTs can be singlewalled or multiwalled and of varying lengths. These pure carbon materials can be chemically modified to enhance hydrophilicity and to enable functionalization via ligands or radiolabels. To modify their surfaces, CNTs have been wrapped in polymeric chains, which can be held in place by high hydrophobic/philic adherence, electrostatic interactions or covalent linkages. Paradoxically, despite their long length (200–300 nm long), it has been shown that covalently functionalized CNTs can be cleared rapidly by renal filtration owing to their small diameter (only 1 nm).

Polymeric nanoparticles
Polymeric NPs are structures composed of block-copolymer chains (with linked “blocks” of different monomers). Rationally designed to self-assemble into spherical- or rod-shaped micelles, these can be radiolabelled by encapsulating a radioactive agent in their core or by labelling the polymer layer itself. Polymersomes are self-assembled vesicles made of block-copolymers forming amphiphilic membranes—replicating the features of a lipid bilayer. The diameter of such particles can be controlled through mechanical extrusion, resulting in sizes as small as 200 nm with a narrow polydispersity index.

Dendrimers are a well-studied family of controlled branched polymer macromolecules. The particles consist of sequential identical groups, a dendron, linked together. The number of these branched groups can be controlled, and therefore particle size can be carefully tailored, offering various degrees of hydrophilicity and/or functionalization. For example, a fourth generation dendrimer, dubbed G4, would constitute four layers of different monomers. Rationally designed to self-assemble into spherical- or rod-shaped micelles, these can be radiolabelled by encapsulating a radioactive agent in their core or by labelling the polymer layer itself. Polymersomes are self-assembled vesicles made of block-copolymers forming amphiphilic membranes—replicating the features of a lipid bilayer. The diameter of such particles can be controlled through mechanical extrusion, resulting in sizes as small as 200 nm with a narrow polydispersity index.

Inorganic nanoparticles
Various approaches have been investigated to formulate stable inorganic NPs. The most common preparations for bioimaging applications are based on the suspension of heavy metals into nanocolloids, which are then stabilized by polymeric, polysaccharide or protein shells. These shells serve multiple functions: to stabilize the NPs, to enable further chemical modification, to reduce adverse biological events and/or to present radiolabelling sites. Among the most thoroughly investigated inorganic NPs are gold NPs (AuNPs; as well as other noble metals such as silver and platinum) and iron oxide NPs (IONPs).

AuNPs can be synthesized with tightly controlled size and aspect ratio (such as spheres or rods) using chemical reduction methods. AuNPs have intrinsic optical properties enabling their use as nanodiagnostic materials for fluorescence detection or Raman spectroscopy of tumour cells. A convenient chemical approach for surface modification utilizes the covalent bonding of Au to thiols, leading to conjugation of polymers (PEG2000-SH), fluorescent probes or disease-targeting molecules, such as peptides, antibodies or nucleic acids.

IONPs consist of an iron oxide core, with a tunable size ranging from several nanometres to microns, which can be synthesized using a range of techniques. The bare iron oxide core is commonly enveloped in macromolecular structures, including human serum albumin, poly lactic acid, or, most frequently, cross-linked sugars such as dextran or chitosan. Functionalized polymeric coatings, including those used for cross-linked iron oxide, offer surface chemistry for conjugation of targeting molecules or chelates for radiolabels or optical/fluorescent probes.

More recently, polymer-coated metallic core NPs formulated with silver or platinum–cobalt core support were radiolabelled with (125I) for AgNPs or (11C) for Pt-based NPs. Similarly, rare earth cationic assembly elements or upconversion nanophosphores consisting of NaYF4, Yb, Er core associated with Gd3+ and radioactive fluorine-18 (18F) were reported for MR-PET multimodality imaging.

**SINGLE PHOTON EMISSION CT AND PLANAR SCINTIGRAPHIC IMAGING WITH NANOPARTICLES**

Single photon emission computed tomography (SPECT) is the most clinically utilized nuclear medicine imaging technique. Generally, it is used for qualitative assessment of cardiac function or detection of disease. However, recent advances have enabled quantitative, whole-body imaging of radionuclide distribution. The most common isotopes utilized for SPECT imaging are the metastable technetium isotope 99mTc, which has a relatively short half-life (6.1 h), and the longer-lived indium isotope 111In (2.8 days) (Table 1). Both radioisotopes present excellent γ emission properties for sensitive, high-resolution imaging, and 99mTc is available from a convenient generator system.

**Chemistries for single photon emission CT nanoparticle labelling**

A wide choice of radiolabelling strategies of NPs is available depending on the quality, compatibility and radiometal of NPs (Figure 1). The most straightforward approach consists in the direct radiolabelling of NPs through the addition of radioisotope during synthesis or utilizing chelator-free methods. Cases of iron oxide radiolabelling with 99mTc-pertechnetate or 111InCl3 trapped in the shell or nanocore demonstrate stable radioNPs. Similarly, coexisting 99mTc-pertechnetate with sodium thiosulfate or human serum albumin proteins forms colloidal suspensions, namely 99mTc-sulphur colloid and 99mTc-nanocolloid. The colloid size may be tuned through filtration. Direct radiolabelling may be achieved for liposomes utilizing PPL electrostatic
interactions or hydrophilicity/phobicity adherence to membranes (Figure 1). However, limited in vivo stability has often been observed. Alternatively, liposomes may be radiolabelled either by extrusion in a radioisotope-containing fluid to entrap the isotope or via radioactive passive diffusion through membranes. The latter consists in the transportation of radioactive hydrophobic chelates through liposome membranes and entrapment of the radioisotope by transchelation in the aqueous cavity. As a result, the radioisotope is efficiently trapped in the intact vesicle (Figure 1).

The most common NP radiolabelling approach with radiometals utilizes a two-step procedure in which chelates are first conjugated to pre-formed NPs, which are then subsequently labelled by mixing with the radioisotope (Figure 2). Although this approach may be chemically challenging to achieve high specific activity, chelation is often a preferred radiolabelling strategy owing to stable radiometal co-ordination with covalent linkage of the radiocomplex and the availability of numerous metal radionuclides (often without requirement of an on-site cyclotron). As an example, liposome surface chelation can be conducted modifying lipids by attachment of chelators on lipid hydrophilic heads or at the end of PEG chains.

Alternatively, NPs can be labelled via covalent radioiodination. Commercially available reagents chloramine-T and Iodogen can be used to oxidize radioiodide into a mixed halogen species, which readily iodoates activated aromatic groups via electrophilic substitution. This reaction is rapid enough for use with even the shortest-lived iodine isotopes. Many amino acid side groups can be labelled, most notably tyrosine. However, if insufficient numbers are present, amines can be covalently linked to phenol via the Bolton–Hunter reagent (Figure 3). Advantages of this chemistry include mild reaction conditions, regioselectivity and efficiency at room temperature, while generating very high specific activity. One limitation is that activated rings (such as phenol) are deiodinated in vivo, resulting in accumulation of radioiodine in the thyroid. However, consumption of iodine [from potassium iodide (KI) or kelp] beforehand will saturate the thyroid with non-radioactive iodine, thus reducing accumulation and exposure.

Pre-clinical development
A significant volume of pre-clinical research has been conducted aiming to develop biocompatible, non-toxic NPs for detection and monitoring of disease. The versatility of chemical and detection approaches afforded utilizing γ-emitting radionuclides has made them a major focus for work with NPs. In this section of the review, we highlight the aspects of single photon-emitting nuclides for planar and SPECT imaging.

Organic nanoparticles for single photon emission CT and planar imaging
Among the earliest reports of radiolabelled NPs are attempts to use liposomal particles encapsulating 111In-complexes to identify tumours. This early work demonstrated the potential

| Isotope | Half-life and most abundant γ energies (MeV) (% abundance) | Radiolabelling methods | Radiolabelled NPs |
|---------|----------------------------------------------------------|------------------------|-------------------|
| 99mTc   | 6.1 h 0.141 (89%)                                       | HMPAO/glutathione      | Liposomes         |
|         |                                                         | Bisphosphonates        | IONPs             |
|         |                                                         | HYNIC                  | Liposomes         |
|         |                                                         | DTPA                   | Dendrimers        |
|         |                                                         | Direct radiolabelling  | IONPs sulfur-colloid |
| 111In   | 2.8 days 0.245 (94%); 0.171 (90%); 0.023 (69%)           | Oxine/nitrilotriacetic acid | Liposomes |
|         |                                                         | CHX-DTPA               | Liposomes         |
|         |                                                         | DOTA                   | Dendrimers        |
|         |                                                         | DTPA                   | AuNPs; liposomes  |
|         |                                                         | Direct radiolabelling  | IONPs             |
| 67Ga    | 3.26 days 0.009 (36%); 0.185 (20%); 0.300 (16%)         | NOTA                   | Cobalt-ferrite NP |
|         |                                                         | DFO                    | Liposomes         |
|         |                                                         | DTPA                   | Liposomes         |
| 125I    | 60.1 days 0.036 (7%)                                    | Tyrosine electrophilic substitution; iodogen or chloramine-T catalysed | Liposomes |
|         |                                                         | Bolton–Hunter reagent  | Quantum dots      |
|         |                                                         | Direct NP radiolabelling | AgNPs AuNPs      |
of this emerging technology, achieving tumour uptake values as high as 18.5% injected activity per gram (%IA/g) of tissue, using neutral-lipid structures. As well, $^{111}$In-labelled liposomes $^{63,64}$ and $^{99m}$Tc-liposomes $^{5,65}$–$^{67}$ have been studied to identify active uptake at sites of infection and inflammation. A focus has been to extend the circulation time and enhance tumour targeting. Liposomes have been grafted with long PEG chains of varying length which demonstrate increased blood residency time up to 24 h p.i vs only 4 h for non-coated liposomes. $^{64}$ Molecularly specific targeting using antibody or peptide attachments to the NP surface has been investigated. Conjugation strategies have focused upon binding these molecules at the end of the PEG chains for reduced steric hindrance at the particle surface. Both immunoliposomes $^{68}$ and peptide-conjugated liposomes $^{69}$ demonstrated selective cell internalization in vitro and enabled tumour detection in vivo. However, greater and irreversible liver and spleen uptake was seen especially for immunoliposomes (30–40% IA/g), with limited gains above passive percent initial activity per gram at tumour tissues.

Polymersomes consist of block copolymers, which are long linear amphiphilic polymeric chains made of a hydrophilic and a hydrophobic portion. They can be tailored for specific applications and have greater stability in comparison to liposomes. Similarly to liposomes, polymersomes were radiolabelled with $^{111}$In, utilizing an
encapsulation approach. \(^{111}\)In-tropolone was incubated with pre-formed NPs that engulfed DTPA. \(^{111}\)In was transchelated and readily trapped by DTPA in the aqueous compartment.\(^2\) This approach resulted in highly stable radiolabelled NPs with no detectable radioactive leakage as the polymer membrane is impermeable to its cargo.

Imaging of dendrimer NPs has focused on the well-characterized PAMAM family. The G4 particle, which presents 64 reactive amines, has been modified with the hydrazinonicotinamide (HYNIC) chelator for subsequent radiolabelling with \(^{99m}\)Tc. These NPs demonstrated significant liver, spleen and kidney uptake.\(^{70,71}\) Similar distribution values have been reported for antibody-conjugated G4 dendrimer targeted to tumour antigens, with increased tumour uptake.\(^{72}\) In comparison, smaller G2 particles possess a faster excretion rate.\(^2\) The G2 particles have been functionalized with antibodies and the common radiometal chelator 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), for labelling with \(^{111}\)In, \(^{90}\)Y or \(^{88}\)Y.\(^{26,73}\) Finally, an alternative dendrimer scaffold of polydiamidopropanoyl (PDAP) functionalized for both radiolabelling and tumour targeting has been used for imaging. PDAP dendrimer was dually targeted to pancreatic cancer cells by peptide and nucleic acid aptamer conjugation, as well as functionalized with the DOTA chelate.\(^{111}\) In-radiolabelling enabled evaluation by biodistribution and planar scintigraphy to show greater uptake for xenografts bearing the targeted epitope.\(^{74}\)

CNTs have been widely investigated as fluorescent imaging and drug delivery platforms. The highly stable carbon–carbon bonds of the CNT can be directly functionalized using harsh chemical syntheses. Utilizing these methods, Das et al reported a dual-modality fluorescent/SPECT (\(^{99m}\)Tc) tumour-targeted multiwalled CNT (MWCNT) for therapy. Folic acid, methotrexate drug and the fluorophore AF-488/647 were conjugated to MWCNT. The construct was then radiolabelled with \(^{99m}\)Tc and a SnCl\(_2\) reducing agent utilizing direct electrostatic interaction with the MWCNT surface. This direct approach resulted in nearly complete \(^{99m}\)Tc-radiolabelling efficiency and was seen to retain 85% of the label over 24 h p.i.\(^{75}\)

Inorganic nanoparticles for single photon emission CT

AuNPs have been directed to sites of disease by functionalization with small peptides such as modified bombesin for prostate cancer\(^{76}\) or cyclic-RGDfK for imaging of tumour angiogenesis\(^{77}\) and imaged with SPECT. These AuNPs were radiolabelled with \(^{99m}\)Tc using HYNIC chelation directly onto AuNPs via thiol binding. The particles sizes varied within 10–20 nm, with visible light absorbing wavelength (550 nm). Upon chemical modification of AuNPs one can use Raman spectrometry to confirm conjugation; providing a facile and robust method to characterize particles. RGD conjugated AuNPs resulted in a modest increase of tumour uptake (3% IA/g) as compared with non-targeted AuNPs (1.5% IA/g).\(^{78}\) \(^{111}\)In-DTPA-AuNP have been used as an imaging and distribution platform to study the effect of size, surface chemistry and PEG chain length on the stability and pharmacokinetics (PKs) of AuNPs. Long PEG chain (5K: 5000 molecular weight) conjugated to theoacetic acid on AuNPs exhibited higher stability as compared with shorter PEG chain length and direct thiol conjugation.\(^{78}\) Furthermore, smaller NP size (20 nm) reduced retention by the RES, prolonging blood residency and circulation time.

More recently Bogdanov et al addressed issues of biocompatibility with AuNPs through surface chemistry modification. The PKs of AuNPs grafted with highly hydrophilic coatings composed of methoxypropylethylene glycol connected to polylysine were investigated. This particular coating was reported to decrease the immunogenicity and as such \(^{99m}\)Tc-methoxyPEG-polylysine-AuNP exhibited long blood residency time (\(t_{1/2} = 17.2\) h) and limited RES and kidney uptake.\(^{79}\) While these efforts have sought to utilize the highly configurable AuNP platform for specific disease imaging with limited background distribution, a remaining limitation is the undefined in vivo long-term toxicity.\(^{80,81}\) Finally, the use of radiolabelled IONPs for imaging will be addressed as a multimodality imaging agent later reported in this review (the Positron emission tomography/MR and single photon emission CT/MR nanoparticles Section).
Clinically applied single photon emission CT nanoparticles for nuclear imaging

Despite broad pre-clinical efforts to develop clinically applicable NPs for SPECT, few formulations have made it to the stage of clinical evaluation nor approval by the Food and Drug Administration (FDA). One successful development is $^{99m}$Tc-colloid currently utilized for intraoperative guidance of lymph nodes resection in patients with melanoma and breast cancer. The first generation of $^{99m}$Tc-colloid was human serum albumin nanocolloid (or $^{99m}$Tc-Nanocoll).82 Albeit approved in Europe for nodal detection, this radiopharmacetical is not approved for use in the United States.83 Instead, a modified formulation of colloid named $^{99m}$Tc-sulfur nanocolloid has been validated for the same medical purpose in patients presenting melanoma, prostate84 and breast cancer.85 Clinical trials of $^{99m}$Tc-sulfur nanocolloid are currently ongoing for lymph node visualization for resection in patients with rectal cancer (ClinicalTrials.gov identifier: NCT02112240) and patients with breast cancer (ClinicalTrials.gov identifier: NCT02287675).

An $^{111}$In-encapsulating liposome formulation, named Vescan, was clinically evaluated using SPECT imaging. Phase 3 clinical trials were conducted with the agent for detection of carcinoma and metastases of prostate, lung and breast cancer.86–89 The same probe was also tested for identification of lesions of Kaposi sarcoma and lymphoma in patients with AIDS.89 On average, the malignancy detection rate was around 70%, which was insufficient for FDA approval at the time (>85%).

Further translational liposomal efforts have focused on the use of $^{99m}$Tc/$^{111}$In radiolabelled PEG-coated liposomes or so-called stealth liposomes. These were developed to result in prolonged blood circulation time to achieve higher tumour to background ratios by enhancing the EPR effect.89,90 Stealth liposomes were clinically tested for the detection of infections and inflammations at low and high lipid dose. It was observed that low dose injection resulted in major radioactive accumulation in liver, spleen and bone marrow, in contrast to high dose remaining in blood circulation up to 24 h p.i. Intestinal radioactive accumulation proved hepatobiliary excretions of the digested particles.91

**POSITRON EMISSION TOMOGRAPHY WITH NANOPARTICLES**

Positron emission tomography (PET) is a sensitive and quantitative tracking tool well suited for *in vivo* NP characterization. It provides relatively high resolution images (3–7 mm depending on scanners) of the radioactive distribution through organs with reduced scatter relative to planar and SPECT imaging. The determination of PK, clearance and toxicity of novel nanostructure designs are prerequisites for full pre-clinical and clinical development of any drug or imaging agent. The inherently quantitative PET modality is therefore ideally suited to study these features.

Positron emission tomography isotopes, physical properties, radiolabelling methods

PET radiolabelling approaches consist of two main strategies: utilizing direct covalent chemistries or co-ordination chemistries.92 Radioisotopes amenable to organic chemistry, including $^{11}$C and halogens like $^{18}$F, $^{76}$Br and $^{124}$I, are usually bound covalently, while radiometals are typically bound via chelation. Recent advances have been made which incorporate radiometals directly into or onto the NP for ligand-free radiolabelling approaches, detailed below. Produced by cyclotron bombardment of solid or gas targets ($^{11}$N, $^{11}$C, $^{18}$F-FDG, $^{64}$Cu, $^{76}$Br, $^{86}$Xe, $^{89}$Y and $^{124}$I) or by generator systems ($^{68}$Ga), these positron-emitting isotopes provide a wide range of chemistries and physical properties for imaging when coupled to NPs (Table 2).

Chemistry for positron emission tomography radioisotopes nanoparticle labelling

NP radiolabelling with positron emitters is equally diverse to that of single photon emitting radioisotopes. Considerations such as the nanostructure composition, stability and chemical functionality are important when selecting a PET isotope. Commonly, the choice of label is set according to the biological mechanism interrogated and the *in vivo* residency time of NPs.97

From a radiolabelling perspective, use of $^{11}$C and $^{18}$F-FDG or the halogens $^{124}$I and $^{76}$Br labelling is most often conducted by covalent binding directly onto the NP surface or to a spacer subsequently associated to NPs97 (Figure 3). Short-lived $^{11}$C and $^{18}$F-FDG require fast and efficient radioparticle syntheses, and necessitate on-site cyclotron facilities, commonly limited to academic settings. These radioisotopes are convenient to define PKs and investigate biological events of short duration. For example, the short-lived $^{11}$C radiolabelling has been conducted by N-methylation of $^{11}$C-methyl iodine to amines grafted on iron oxide surface.41

$^{18}$F-FDG is the most commonly used PET isotope in the clinic as either $^{18}$F-FDG-NaF for bone scanning or as $^{18}$F-FDG. Its common use has led to the development of an array of chemistries for labelling, many of which have been adapted and applied to NPs. Fluorination may be conducted by: nucleophile substitution of leaving groups such as aliphatic tosyle98 or as tert-butyli-silicon construct.99 With minimal chemical modification, it is also possible to introduce $^{18}$F-FDG by direct binding to the NP, exploiting fluoride’s reactivity towards aluminium oxide or hydroxyapatite.100 A major recent development in the field of radiofluorination has been the discovery of stable and efficient co-ordination with aluminium–1,4,7-Triazacyclononane-N,N',N''-triacetic acid (NOTA) complexes.101 While only used at this time to label small molecules and peptides, this method may be applied in the future to radiolabel NPs. However, issues associated with the high temperature, organic solvent and low pH needed for radiolabelling may preclude widespread adoption. As previously described, phenols can be radioiodinated utilizing iodogen or chloroamine-T as catalysts. Phenols are available in Bolton–Hunter reagent102 and tyrosines in peptides13—both of which have been connected to nanostructures as a common iodination strategy (Figure 3).

Stable co-ordination chemistry is a pre-requisite for accurate *in vivo* use of probes modified with PET radiometals. Chelation is often achieved utilizing a two-step procedure in which chelators are first conjugated onto the NP surface and then subsequently radiolabelled. DOTA has been widely used to label NPs because it is capable of stably binding various metals, and bifunctional
reactive DOTA is commercially available. 102 64Cu-DOTA in particular has been widely utilized to visualize NP distribution, including for liposomes, 103,104 AuNPs 105 and IONPs. 106 However, DOTA appears to be an unstable chelator for 64Cu 107 and also requires heating to gain efficient labelling yield. Subsequent advances have seen NPs prepared with TETA; 23 DO3A 108 or other innovative ligands such as BAT58 and bisphosphonates 109 resulting in more stable radiocomplexes (Figure 2).

The recent improvement in methods to produce, purify and label 89Zr to monoclonal antibodies for pre- and clinical investigation has made this radiometal of particular interest for the NP community. The half-life of 3.8 days is well suited to the aforementioned immunoglobulins, as well as longer circulating NPs. The desferioxamine (DFO) chelator has been described as the ligand of choice for immuno-PET imaging (Figure 2). However, recent developments have opened a debate whether DFO is sufficiently stable for 89Zr use in vivo, 110 and alternative chelate design is an active area of research. 111 In addition, direct radiolabelling of NPs using chelator-free approaches have also been investigated with liposomes, 6 silica 112 and IONPs. 113

### Pre-clinical development overview/role in drug development

11C, 18F and radiohalogens covalent radiolabelling to nanoparticles

Rapid radiolabelling of Al2O3 NPs was reported utilizing an elegant method of NP radioactive activation generating 13N-enriched NP. 114 This approach enabled immediate characterization of NP PKs comparing various NPs size (from 10 nm to 10 μm diameter). This study showed that the smaller the NPs, the higher the chance of urinary excretion with significant bladder uptake. Increasing particle size resulted in accumulation in liver, spleen and lungs. 114

To the best of our knowledge, the only report of 11C-labelling of NPs has been accomplished by 11C-methylation of IONPs allowing for immediate PK determination. Minutes-long half-life of 11C limits its use for extended radioactive monitoring. However, further visualization of these particles was defined

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### Table 2. Positron emitters for nanoparticle imaging applications

| Isotope | Half-life and most abundant energies (MeV) (% abundance) | Radiolabelling methods | Radiolabelled NPs |
|---------|----------------------------------------------------------|------------------------|-------------------|
| 13N     | 9.96 min, $\beta^+$: 1.19 (99%), $\gamma$: 2.22 (0.182%)  | Radioactive activation | Al2O3            |
| 11C     | 20.36 min, $\beta^+$: 0.960 (99%)                        | Methyl iodine substitution | IONPs          |
| 18F-FDG | 109.8 min, $\beta^+$: 0.635 (100%)                       | Cu-catalysed click chemistry between 2-[18F]-fluoroethyl azide and alkyne-NHS spacer, Michael addition of SiFASH spacer, Direct encapsulation of 18F-FDG | Dendrimers, AuNPs, 18F FDG-liposomes |
| 78Br    | 16.0 h, $\beta^+$: 3.980 (26%), $\gamma$: 0.559 (74%), 0.657 (16%), 1.854 (15%) | Nucleophilic substitution on tyrosine | Dendrimers |
| 124I    | 100.2 h, $\beta$: 1.54 (11.7%), 2.14 (10.8%), $\gamma$: 0.603 (63%), 1.691 (10.9%), 0.723 (10.4%) | Nucleophilic substitution on tyrosine catalysed by iodogen | Dendrimers, Silica dots |
| 68Ga    | 67.71 min, $\beta^+$: 1.899 (89%), $\gamma$: 1.077 (100%) | NOTA chelation, Direct radiolabelling | AuNPs, IONPs |
| 64Cu    | 12.70 h, $\beta^+$: 0.578 (37%), $\beta^-$: 0.651 (18%), $\gamma$: 0.511 (36%), 0.007–0.008 (34%) | DOTA, TETA, BAT, DO3A chelation, Bisphosphonate, Direct radiolabelling | Liposomes, QDs, AuNPs, Polymeric micelles, Solid lipid NPs, QDs, IONPs, solid lipid NPs, IONPs, AuNPs |
| 89Zr    | 78.4 h, $\beta^+$: 0.897 (77%), 22.6%, $\gamma$: 0.909 (99.9%) | Direct radiolabelling, DFO chelation | Liposomes, IONPs, AuNPs, SWCNTs |
| 86Y     | 14.74 h, $\beta^+$: 1.2 (11.9%), 1.5 (5.6%), $\gamma$: 0.177 (83%), 0.627 (32.6%), 1.153 (30.5%) | DOT A | SWCNTs |

$\varepsilon$, electron capture; $\beta^-$, beta decay; $\beta^+$, positron; AuNPs, gold NPs; IONPs, iron oxide NPs; QDs, quantum dots.
utilizing the particles’ intrinsic MR contrast. It should be stated however, that the short-lived isotope has the advantage of limiting prolonged radioactive exposure.41

Radioactive fluorination has been undertaken on many NP species. Direct fluorination can be employed when working with aluminium oxide or hydroxyapatite NPs. This radiolabelling process is based upon 18F exchange with hydroxides carried by aluminium oxides or hydroxyapatites. In vitro serum assays demonstrated high stability, however, evaluation in mice showed increasing bone uptake owing to defluorination, after predominant lung and liver uptake.100

Liposomes have been fluorinated using a variety of strategies. Incorporation of readily made radioactive constructs in formulation is perhaps the most straightforward. Reports of 18F-FDG and radiofluorinated drugs encapsulated in vesicles showed enhanced drug delivery and enabled imaging of liposomes.115,116 However, radiation exposure during liposome extrusion and low encapsulation efficiency are the areas of concern. Fluorination of the lipid constituents used to produce the liposomes can be labelled with improved overall efficiency, but similarly, radiation exposure remains a challenge.98 Alternatively, Urakami et al have proposed 18F-radiolabelling utilizing preformed liposomes. Single chain lipid constructs were radiofluorinated by tosy1 substitution and subsequently incubated with preformed liposomes. This approach resulted in high incorporation efficiency (>80%) and fair in vitro stability in serum proteins.117

18F-radiolabelling of AuNPs has been accomplished exploiting the thiol reactivity of Au-surface. Maleimide functionalized PEG grafting to AuNPs were reacted with thiol substituted 18F-silicon prosthetic group (Si-FA-SH) through [1,4]-Michael addition. The silicon fluoride construct was produced by fast 18F isotopic exchange with a t-butyl silicon construct.99 The resulting carboxylated AuNPs of <3 nm diameter were administered to rats and exhibited transport across the blood brain barrier.118 A similar process to radiolabelling preformed particles has been employed with Cu-catalysed click chemistry to image dendrimers. A succinimidyl alkyne construct was assembled to react with 18F-fluorinated azide prosthetic group. Amine-terminated generation-6 dendrimers were then conjugated with 18F-labelled spacer resulting in fast and efficient radiolabelling.119

Use of halogen radioisotopes has been explored as well for dendrimers radiolabelling. Dendrimers have been modified with tyrosine allowing for radiohalogen substitution with 125I or 70Br. Tyrosine amines were attached to RGD for specific angiogenesis imaging application.120 Similarly, Benezra et al121 utilized RGD tyrosine as a site for iodination of silica dots for 124I-PET imaging of human melanoma.

Co-ordination chemistry and electrostatic interaction for nanoparticle radiolabelling

Positron emitting radiometals including 68Ga, 64Cu, 68Y and 89Zr can be bound to NPs by electrostatic interactions or co-ordination chemistry. Generally, 68Ga is used to probe short biological phenomena, while 64Cu, 68Y and 89Zr enable longer investigation and identification of NP body residency time.

Various AuNP surface chemistries and sizes have been compared to investigate short-term PKs using a 68Ga-NOTA radiolabelling approach. NOTA was conjugated using a long or short arms to glycopeptide-functionalized AuNPs and subsequently radiolabelled by mixing with 68Ga.122 Short spacer conjugation and peptide modification exhibited an increased renal clearance as compared with other formulations. Furthermore, this preparation enabled a higher brain uptake, showing the potential of AuNPs for neuroimaging. AuNPs were also radiolabelled utilizing 64Cu-DOTA to provide a long-term investigation of distribution out to 44 h p.i.123,124 Grafting AuNPs with 2K and longer PEG chains significantly increased NP diameter (to 20–50 nm) resulting in enhanced liver and spleen uptake.123

Direct incorporation of the radiolabel within the nanostructure can also be conducted. Human serum albumin-stabilized gold nanoclusters were doped with 64Cu to generate dual-modality near infrared (NIR) fluorescence and PET-capable ultrasmall particles (7 nm), which were then characterized in vivo. Tumour targeting was achieved with 15% IA/g 24 h p.i. but notable liver uptake (up to 30% IA/g) was noticed as early as 1 h p.i.124,125 AuNPs have been tracked through conjugation of 89Zr-radiolabelled mAb.89Zr-tracking enables visualization up to 168 h p.i.125,126

Liposomes exhibit long in vivo circulation, especially when grafted with PEG chains.27 For accurate characterization, they require stable radiolabeling modification, with long radioisotopes. PET imaging of liposomes has therefore been undertaken utilizing 64Cu and 89Zr. Similar to SPECT radiolabelling, loading radioisotopes into the aqueous compartment of the particles has been tested. Here, liposomes were formulated with encapsulated DOTA to enable 64Cu incorporation by using the lipophilic transporter hydroxyquinoline. These 64Cu-liposomes have then been used for both tumour and inflammation imaging.127,128 Aminofunctionalized AuNPs have been synthesized by conjugation to octreotide6 or octreotate103 Direct 64Cu-radiolabelling of the liposomal surface has been achieved using with BAI, a stable chelator of 64Cu.130 The chelator was conjugated to the end of PEG chains and radiolabelling was conducted on preformed NPs. 64Cu has enabled extended visualization of NPs up to approximately 48 h p.i.

For longer imaging, 89Zr-labelling has been used.131 89Zr-radiolabelling of liposomes was conducted utilizing DFO chelation of PEG-functionalized lipids31 or by direct embedment in lipid membranes.13 The latter provides a straightforward approach with minimal modification but produces moderately stable particles, with significant bone uptake as compared with DFO-conjugated liposomes. Tumour-targeted PET liposomes have been synthesized by conjugation to octreotide or octreotate115 (the C-terminal threonine in octreotide being replaced with threonine to yield octreotate). These peptides target a subset of the somatostatin receptors (sstr2), which is overexpressed on neuroendocrine tumour cells.135 Although octreotide receptor affinity for the sstr2 receptor is known to be higher than DOTA-[Tyr3] octreotide (IC50 = 1.5 vs 14 nM, respectively132), octreotate-liposomes did not show greater tumour-specific contrast in comparison with octreotide-liposomes. Peptide-conjugated liposomes resulted in higher liver and spleen uptake as compared with control PEG-liposomes, a result of increased RES filtration. In additional to liposomal approaches, polymer-based particles
have been closely investigated. Micelles made of block copolymers were engineered and radiolabelled with $^{64}$Cu-TETA. Folate bound to the end of PEG$_{1,6K}$ chains were conjugated to NPs. The majority of uptake was revealed in the lungs, liver and spleen as early as 10 min p.i. Tumour targeting was, however, not achieved when compared with controls. Taken together, these results raise interesting question about the overall ability of targeting ligands to direct (relatively) large NPs structures to disease sites through active uptake mechanisms.

In the absence of targeting ligands, PET radiolabelling has afforded the ability to image the effect of surface coating for NP distribution. Here, $^{64}$Cu-DOTA-labelled CdSe QD-PEG$_{2000}$ was compared to similarly radiolabelled non-coated QDs. Delayed uptake in the liver and spleen was noted when QDs were PEG-shielded. Smaller-sized InAs QDs have also been investigated with PEG shielding and peptide modification, which demonstrate increased renal clearance when peptides were conjugated, while blood circulation was extended when PEG coated.

Dextran-formulated polysaccharide NPs have been radiolabelled with $^{89}$Zr-DFO to track macrophages. The sugar polymer, synthesized into spherical nanostructures, was modified for amino functionalization, providing a site for conjugation of fluorescent probes and $^{89}$Zr-radiolabelling with DFO. This labelling strategy was used to investigate the effect of size and functionalization of dextran NPs in vivo, where it was noted that the smaller the size, the faster the clearance from blood. Similarly, the more hydrophilic the coating, the faster the blood clearance. Little uptake was noted in background organs, as NPs distributed mostly into lymph nodes, liver and spleen. The tracking of tumour-associated macrophage was further confirmed by fluorescence flow cytometry of digested tumour tissues.

Covalently functionalized, single-walled CNTs have also been evaluated using PET radiometals. CNTs were conjugated with DOTA for $^{86}$Y labelling or with DFO for $^{89}$Zr labelling. These strategies have provided insight into both the short and long-term distribution characteristics of these NPs. Surprisingly for their long length, renal excretion was the major pathway of CNT clearance. Furthermore, fluorescent tagging of the same particles enabled investigators to focus on the uptake mechanism in renal tubule cells. This radiosensitive cell is relevant for radiotherapy applications utilizing an $\alpha$-particle emitting $^{225}$Ac-CNT construct evaluated to treat a colon cancer model. It was demonstrated that CNTs were located on the basal compartment of the kidney cells, enabling use of this potent therapy with minimally observed toxic effect.

Translation of nanoparticles for positron emission tomography imaging

To date, few PET-radiolabelled NPs have been evaluated clinically. C-dots are perhaps the pioneer PET-labelled NPs and are currently undergoing translation in Phase I clinical trial to evaluate safety and efficiency (ClinicalTrials.gov identifier: NCT01266096) (Figure 4). C-dots are constituted of an inorganic core with a Cy5.5-fluorescent dye, stabilized by a silica shell. The formulation used in clinical investigation is PEG coating to avoid RES uptake and modified with a $^{124}$I-labelled-cRGD peptide for tumour-targeting to the $\alpha$$_2$$\beta$$_1$-integrin, expressed on malignant neovascularure. At this time, evaluation of the compound in five patients with melanoma and some with malignant brain tumours have been published. Whole body patient imaging demonstrated uptake in the gallbladder, heart, intestines and bladder as well as localization to the tumour (Figure 4).

The in vivo residency time in humans displayed both immediate- and delayed-clearance phases with measured values of $t_{1/2} (1) = 3.75$ h and $t_{1/2} (2) = 48$ h, respectively. The mechanism of clearance was through the kidney, which produces the advantage of fully removing the particles (rather than, for example, decomposition in the liver). Complete PKs, organ dosimetry and follow-up of NP metabolites in plasma and urine were evaluated. Ultimately, this multimodality imaging NP platform may facilitate tumour detection utilizing PET and ease tumour resection utilizing fluorescence-guided surgery. Furthermore, the initial evaluation of the agent in patients with melanoma is being expanded to guided lymph node imaging in patients with head and neck, melanoma, prostate and cervical cancer (ClinicalTrials.gov identifier: NCT02106598). This trial focuses on utilizing a single nanostructure for two or more imaging modalities, which will be explored in the following section.

**MULTIMODAL IMAGING WITH RADIOLABELLED NANOPARTICLES**

With the emergence of multimodality imaging scanners such as PET, SPECT/CT and PET/MR, also came pre-clinical-developments in the form of probes. Considering the variety and customization of NP compositions and their diversity in surface chemistries, nanostructures are ideal platforms for multimodality imaging application.

A major driving force for the development of NP-based imaging agents and more broadly for the application of NPs in biomedical science is the potential for multiplexing. As the synthesis, modification and characterization of NPs has improved in the past two decades, intense effort has been focused on their use as multifunctional platforms. Specifically for diagnostic and disease monitoring applications, signal generation in more than one imaging modality is of use to take advantage of the strengths of each modality. CT and MR provide important anatomical detail, emerging optical imaging technologies generate high resolution and post-mortem histological verification, while PET and SPECT enable high sensitivity of detection of metabolism and ligand-targeted information. Taken individually, each modality presents its own advantages and limitations. Combining two or three imaging modalities within the same NP formulation allows for the exploitation of each modality’s advantageous properties.

By virtue of the physical properties of their constituents IONPs, AuNPs and QDs feature original imaging contrasts for MR, CT or optical imaging, respectively. NP radiolabelling opens a route towards novel diagnostic approaches utilizing simultaneous contrasts in multiple modalities. An attractive prospective of multimodality imaging utilizing NPs is the application for image-guided surgery facilitating sensitive deep tissue detection of disease by PET, followed by precise tissue resection by optical contrast.
Positron emission tomography/MR and single photon emission CT/MR nanoparticles

The complementary nature of different imaging methods has been a major force driving improvements in clinical radiology and pre-clinical research. Most notably, the fusions of PET/CT and SPECT/CT have enabled researchers and clinicians to visualize functional and anatomical details simultaneously. The overriding advantage is the exact localization of sites of radiotracer uptake. In the last decade, advances in imaging scanner technology resulted in the emergence of novel devices combining complementary imaging modalities, such as PET/MR and PET-SPECT/CT. PET and SPECT are ultrasensitive, quantitative imaging techniques that determine functional mechanism, whereas CT and MR confer detailed morphological and anatomical information. The development of multimodality imaging probes may be of tremendous benefit for defined medical applications. For example, radiolabelled IONPs may increase the sensitivity of lymph node detection by nuclear imaging, to assist disease detection by anatomical MRI in humans.135

Specific radiofrequency (RF) pulse sequences are used in MRI to visualize contrast between adjacent tissues. These RF sequences are designed to accentuate the longitudinal (also known as $T_1$) or transverse ($T_2$) relaxation times of the protons in a particular tissue. Pulse sequences vary the excitation and time of readout in order to capture the $T_1$ and/or $T_2$ signal of a particular tissue. The $T_1$ or $T_2$ signal is dependent on several factors which include proton density and the biochemical nature of the hydrogen atoms present in that organ or liquid. Contrast agents are applied to locally enhance the signal in a given imaging protocol. While all contrast agents possess both $T_1$ and $T_2$ effects, contrast agents are generally characterized by their dominant contrast mode. $T_1$ weighted contrast agents increase the rate at which protons realign with the applied magnetic field (thereby increasing signal locally in $T_1$ weighted imaging). Conversely, $T_2$-contrast agents (such as IONPs) function by enhancing the loss of coherent precession of local protons (thereby decreasing signal locally in $T_2$ weighted imaging). Commonly, iron oxides are referred to as $T_2^*$ contrast agents, a term that refers to the observed $T_2$ signal, shortened by unavoidable magnetic field inhomogeneities and other field distortions.

Combining PET with $T_1$ weighted Gd-engineered NPs have been prepared with radiolabelled liposomes ($^{89}$Zr, $^{64}$Cu and $^{111}$In) formulated with Gd-DTPA/DOTA-lipids.47,49 Another reported strategy describes inorganic upconversion phosphors, incorporating rare earth metals and Gd into a nanostructure. These NPs produce detectable spin-lattice MR contrast enhancement and are radiolabelled with $^{18}$F for dual PET/MRI.44

The $T_2$-contrast producing IONP agents have been widely investigated for combined SPECT and PET with MR. These particles may be ferro- or superparamagnetic, enabling contrast in MRI through the production of localized magnetic field inhomogeneities.135 Formulations of IONPs (free of a radiolabel) have been approved for clinical use and are under investigation for a range of disease detection applications including breast, pancreatic, thyroid and head and neck cancer (ClinicalTrials.gov identifier: NCT01927887). Using these NP platforms, IONPs are an inherent multimodality imaging agent for dual PET/MR ($^{89}$Zr-labelled ferumoxytol40 or SPECT/MRI ($^{111}$In-labelled IONP,49 as well as trimodality imaging MR/NIR/PET or SPECT when conjugated to fluorescent near-infrared optical agents (see the Radiolabelled fluorescent nanoparticle section). Radiolabelled IONPs with SPECT imaging isotopes using direct labelling or doping approaches have produced $^{99m}$Tc-labelled and $^{111}$In-incorporating IONPs.47,48 Alternatively, to preformed particles, $^{99m}$Tc-bisphosphonate can be used to radiolabel IONPs.141 SPECT and $T_2$-MR, and $^{99m}$Tc-IONPs have demonstrated sensitive identification of lymph nodes in rats.

Fast and efficient click chemistry strategies have also been leveraged to produce $^{18}$F-radiolabelled IONPs. $^{18}$F-labelling was conducted with Huisgen cycloaddition of a fluorinated alkyne to azide-modified NP surfaces.142 This PET/MRI agent permitted macrophage tracking in aortic aneurysms in mice.143 IONPs

Figure 4. Whole-body positron emission tomography (PET)-CT imaging of $^{124}$I-cRGDY-polyethylene glycol–Cornell dots. Intravenous injection of $^{124}$I-cRGDY-PET-C-dots followed by repeat imaging in a Phase I safety and feasibility study (ClinicalTrials.gov identifier NCT01266096). Representative whole-body image of (a) CT, (b) PET at 4 h and (c) PET/CT at 4 h and (d) at 24 h. (e) Corresponding fluorine-18 fludeoxyglucose PET/CT image of hepatic metastasis in (a); (arrow). Colour and grey scales reflect PET standardized uptake value. Reproduced from Phillips et al17 with permission from American Association for the Advancement of Science.
have also been radiolabelled for PET imaging with radiometals such as \(^{68}\)Ga (doped), \(^{64}\)Cu (doped or DOTA/bisphosphonate chelated), \(^{109,145,146}\) or \(^{89}\)Zr (doped or DFO chelated).\(^{113,146}\) The high radioactive concentration per NP, or specific activity, is propitious for Cerenkov imaging, further presented in the last section of this review.\(^{140,144}\)

Radiolabelled fluorescent nanoparticles

Combination of nuclear and optical imaging is beneficial considering the complementary advantages of each modality. Nuclear imaging is quantitative, sensitive and does not have any tissue-depth limitation, while optical imaging can be sensitive and is applicable to real-time intraoperative contexts but is only amenable at shallow depth or in exposed tissues.\(^{147}\) The modalities may therefore be combined to provide a means to simultaneously detect deep tissues, for subsequent guided resection of sites of interest, often lymph nodes or disease foci. Nuclear imaging scans localize the zone to operate, and optical image guidance helps to complete precise resection of tumours or metastases.

Radiolabelled fluorescent NPs demonstrate useful features for histopathological evaluation of diseased tissues. As well, this approach provides a means for high-resolution cellular and subcellular in vitro study of NPs interaction with cells by fluorescence microscopy.\(^{148}\) Some NPs, such as IONP have been conjugated with fluorophores (Cy5; VT680)\(^{149,150}\) and radiolabelled (with \(^{64}\)Cu or \(^{18}\)F) for tracking of macrophages at inflammation sites\(^{150}\) or for detection of intracardiac macrophages.\(^{143}\) Other NPs such as QDs have intrinsic fluorescent features,\(^{146,151}\) that afford fluorescent imaging of the radiolabelled particles. As mentioned above, the C-dot PET NP currently undergoing clinical evaluation encapsulates Cy5.5 fluorophore.\(^{17}\) It is hoped that this will contribute to multimodal imaging applications using this NP platform to non-invasively detect disease by PET, followed by fluorescence-guided resection.

Cerenkov radiation imaging with nanoparticles

As an alternative to radiolabelling of NP platforms, which present contrast in other modalities (for example, radiolabelling fluorescent QDs), the radioisotope itself has the potential to enable multimodal nuclear/optical imaging. Cerenkov radiation (CR), named for Dr Pavel Cerenkov’s characterization of this phenomenon, is a physical process by which high-energy charged particles produce ultraviolet- and visible-wavelength light when travelling through dialectric media (such as water or tissues).\(^{152,153}\) Several conditions must be met for the production of this light; most critically sufficient velocity of the charged particle at super-relativistic speeds in the media. In water, with a refractive index of 1.33, the required velocity threshold is 263 keV and is met by many of the \(^{125}\)I emitting particles used for PET imaging and radiotherapeutic radionuclides such as \(^{90}\)Y, \(^{111}\)In, \(^{177}\)Lu and \(^{225}\)Ac.\(^{154}\)

At this time, applications in CR optical imaging have predominantly used PET isotopes.\(^{155,156}\) NPs provide a platform to achieve a high number of radionuclides per structure, which may be essential in many CR imaging applications because of the low number of visible photons produced per disintegration.\(^{154}\) Tyrosine-conjugated polymer-coated IONPs were radiosiodinated with iodogen beads and \(^{124}\)I, to produce a probe for optical/PET/MRI.\(^{158}\) Following dermal injection in the footpad, lymph nodes can be identified using optical, PET and MRI. Optical/MRI of nodes with the short-lived \(^{68}\)Ga bound magnetic NPs using a direct labelling method was demonstrated.\(^{144}\) Systemic imaging of optical/PET/MR liposomes has also been achieved, using a radiosiodinated (\(^{124}\)I) lipid analogue constituent.\(^{159}\) NPs emitting \(^{198}\)Au (clinically used as a colloid for local treatment of arthritis\(^{160}\) and meningosis\(^{161}\)) has been labelled to AuNPs of controlled shapes and subsequently optically imaged by CR emissions\(^{162}\) and evaluated to treat a breast cancer model in mice.

While nuclear imaging provides exquisite sensitivity unmatched for deep tissue whole-body molecular imaging, the physical principles of PET preclude imaging of multiple tracers simultaneously (as is commonly done with fluorescent agents) or the construction of activatable contrast agents. Such activatable probes respond to molecular or cellular events to enable readout of physiological or disease processes. CR allows for both multispectral and activatable nuclear probes to be evaluated. As recently investigated, implicated enzymatic activity was visualized in aggressive disease of a breast cancer model.\(^{163}\) Here, AuNPs were conjugated to a fluorophore via an enzyme-cleavable linker. When bound to the particle, the fluorescence of the fluorophore is quenched, but after cleavage can be excited by CR and the fluorescence spectrally distinguished. A similar distance-dependent activatable strategy has utilized \(^{64}\)Cu-conjugated DNA sequences of varying length bound to QD. Distance-dependent spectral shifting of the CR via the fluorescent QD was noted.\(^{164}\)

In an alternative strategy, the Cerenkov signal can be quenched by absorbing NPs. For example, NPs can be directed to sites of disease, which are concomitantly targeted with \(^{18}\)F-FDG. In the event of co-localization, the CR signal is diminished, enabling combined optical and nuclear imaging to enhance the information content from a single study.\(^{165}\)

The continuous spectrum of light produced by the CR effect is weighted to the short wavelength UV and blue. NIR fluorescent NPs coupled to CR-producing radioisotopes absorb the blue light of the CR and emit red-shifted fluorescent light. Red and NIR light can travel deeper through tissue for more efficient detection. QDs have been mixed with radionuclides for this effect, which also enables multiplexing (spectral distinction of different probes simultaneously).\(^{156,163,166,167}\)

CONCLUSIONS AND FUTURE DIRECTIONS

Biomaterial and chemical engineering developments have created opportunities for multifunctionalized NP platforms across many scientific disciplines. The significant medical potential of these NPs has generated intense interest in the molecular imaging and radiology communities. Careful construction and evaluation of these NP probes using nuclear imaging methods provides considerable insight into their in vivo fate and their prospective utility in research and clinical application. These discoveries have demonstrated how size, charge, shape and surface chemistry are critical criteria to consider for NP biocompatibility, safety and clearance. Ultimately, few nanoformulations have made
Radiolabelled NPs have been investigated for single-modality SPECT and PET imaging, as well as multimodality nuclear MR and optical imaging in a range of pre-clinical models. These accomplishments may open new paths and technical developments for the ultimate goal of facilitating diagnosis of disease and precise medicine.

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